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National Wildlife Refuge System

Site-Specific Protocol for Monitoring Abundance of Río Yaqui Fishes in Streams

San Bernardino and Leslie Canyon National Wildlife Refuges

Survey ID Number: FF02RASB00-059

Version 1.1

ON THE COVER
A stream located at El Coronado Ranch near San Bernardino and Leslie Canyon National Wildlife Refuges. Fish (counter-clockwise from bottom left): Yaqui chub, Yaqui topminnow, and beautiful shiner.
Photographer(s): D.R. Stewart and W.R. Radke.

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Protocol Title: Site-Specific Protocol for Monitoring Abundance of Río Yaqui Fishes in Streams

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Version[1]	Date	Author	Change Made	Reason for Change

[1] Version is a decimal number with the number left of decimal place indicating the number of times this protocol has been approved (e.g., first approved version is 1.0.; prior to first approval all versions are 0.*x*; after first approval, all minor changes are indicated as version 1. *x* until the second approval and signature, which establishes version 2.0, and so on).

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[3] Signature signifies approval of a site-specific survey protocol by the Zone I&M Biologist.

[4] Signature by Regional I&M Coordinator signifies approval of a protocol framework to be used at multiple stations within a Region.

[5] Signature by Buenos Aires, Leslie Canyon, and San Bernardino NWRs Refuge Complex Manager signifies approval of a protocol framework to be used at the Refuge.

[6] Signature by the Arizona/ New Mexico NWRs Refuge Supervisor signifies approval of a protocol framework to be used at the Refuge.

[7] Signature by National I&M Coordinator signifies approval of a protocol used at multiple stations from two or more Regions.

Survey Protocol Summary

This survey protocol provides standardized methods for monitoring smaller-bodied Río Yaqui fish species (beautiful shiner *Cyprinella formosa*, Yaqui chub *Gila purpurae* and Yaqui topminnow *Poeciliopsis occidentalis sonoriensis*) and the Mexican longfin dace *Agosia* sp. in streams located in and around San Bernardino and Leslie Canyon National Wildlife Refuges and El Coronado Ranch (West Turkey Creek, AZ). The beautiful shiner is federally listed as threatened, and both the Yaqui chub and Yaqui topminnow are federally listed as endangered (USFWS 1994). As it relates to the three Río Yaqui fish species, they are found nowhere else in the United States. Their status remains undefined in Mexico. Therefore it is critical to reliably estimate "true" abundance of these populations as a means to define their status, assaying trends through time, and evaluating the efficacy of protection and management efforts toward their recovery. Given that the beautiful shiner is found to be rare in streams, occurring in low numbers and not encountered annually, it will not be a focus of this survey. Additionally, the service also collects information on the Mexican longfin dace to share to the Arizona Game and Fish Department.

The annual abundance of Río Yaqui fishes in streams has been enumerated using depletion experiments since 2004. However, a formal written protocol, including survey objectives, survey methods, sampling frame, data analyses, and reporting procedures, was never completed. The long-term monitoring of each species relied on a technique that is designed to account for detectability and has been in place for several years but improvements to the technique are needed. For example, behavior of Río Yaqui fishes and their specific habitat requirements were not considered with the traditional method (Stewart et al. 2019). Moreover, the environmental conditions (species habitat requirements) in most cases can negatively affect their detectability, and thus in the past one was not able to disentangle changes in observed abundance from changes in detectability due to environmental conditions that may have made it easier or more difficult to capture each species (Stewart et al. 2019). Depletion sampling also requires constant effort defined by similar number of "on-time" seconds per pass. We know from the historical data that the number of "on-time" seconds per pass at times varied significantly between and among passes, with the first pass being slower (e.g., 321 seconds) to complete than the remaining two successive passes (e.g., 180 seconds). Additionally, the traditional statistical method used to generate estimates of abundance and detection probability is an invalid approach, as it fails to account for varying detection probability, does not consider more than one stream reach at a time and requires analysis for each stream reach and year separately. As a result, we implement improvements to the previous sampling technique and the associated analytical methods to increase the detection of each of the Río Yaqui fishes, prevent underestimation of their true abundance, and ensure that the abundance estimate produced directly relates to true abundance and reliably track changes in abundance/ status over time (Stewart et al. 2019).

From 2015-2017, the U.S. Fish and Wildlife Service re-evaluated the objectives and methods of the traditional technique and launched a multifaceted effort to improve the survey methods (Stewart et al. 2019). The standardized survey methods originated from a series of simulation studies to evaluate how true abundance and the number of depletion passes affected relative bias in the abundance estimate under two scenarios: constant detection probability across successive passes and varying (declining) detection probability across successive passes. Additionally, we

used a series of field validation experiments to complement our simulation analyses by establishing known populations, implementing the new standardized survey protocol, used a new presentation of a hierarchical Bayesian multi-population negative-binomial mixture model that is designed to account for detection probability that varies across successive passes and also provides spatially-distinct estimates for both abundance and the detection probability through time (Stewart et al. 2019). Moreover, these models also include how habitat affects the detection probability and their abundance, which can be used to provide a better understanding of Río Yaqui fish resource use, which will guide recovery and conservation efforts.

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This protocol is available from ServCat [https://ecos.fws.gov/ServCat/Reference/Profile/108477]

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Narrative

Element 1: Introduction

Background

The San Bernardino and Leslie Canyon National Wildlife Refuges (NWR) were created in 1982 and 1988 for the purpose of conserving endemic fish species native to the Río Yaqui basin (USFWS 1994). Growing threats from groundwater pumping, land use changes, loss of connectivity due to barriers, introduced species, and altered stream flows have exacerbated species declines (Stewart et al. 2017a). Four of the eight endemic species remain. The beautiful shiner *Cyprinella formosa*, Yaqui topminnow (*Poeciliopsis occidentalis sonoriensis*) and Yaqui chub (*Gila purpurae*) are US federally listed as endangered, and Yaqui catfish (*Ictalurus pricei*) are US federally listed as threatened with extinction (USFWS 1994). Our focus centers on Yaqui chub and Yaqui topminnow, which continue to thrive in spring-fed ponds and streams found on and around these Refuges. The beautiful shiner is found to be rare in streams, occurring in low numbers and not encountered annually, and thus it will not be a focus of this survey. The survey can be amended in the future to include beautiful shiner in the event that their encounter histories increase. Additionally, the service also collects information on the Mexican longfin dace (*Agosia* sp.) to share to the Arizona Game and Fish Department, and thus this species was included as a species to monitor in this survey protocol.

In 1994, the U.S. Fish and Wildlife Service finalized a recovery plan with the intent to restore and create self-sustaining populations including downlisting criteria. These criteria range from securing and protecting San Bernardino Valley aquifers, eradicating all non-native fish species, and protecting critical habitats where these species occur or have been reintroduced. The criteria for Yaqui chub and Yaqui topminnow are specific to effectively managing only those populations in the US. However, one of the primary criterions across all species requires the establishment of populations that remain secure, which requires monitoring the status of these fish populations and their occupied habitats to determine how each may change in the future (USFWS 1994).

Estimating abundance of rare and endangered species remains vital for evaluating species conservation status and determining if they are “secure”, assaying their trends through time, and evaluating the efficacy of protection and management actions. Monitoring also provides a tool to determine if a species has achieved downlisting criteria. Therefore, it is critical that the USFWS frequently and objectively critique the methods we use to measure the effects of conservation and management, especially when monitoring rare and endangered species. In doing so, we ensure that the highest quality scientific data and information is collected to support the mission of the Department (Department of the Interior 2011).

Long-term monitoring using a technique designed to account for detectability has been conducted since 2004, however, improvements to the technique were needed (see Appendix A for more details). Below, we have identified additional methods to improve on the existing approach. Methods described in this protocol increase the reliability of the information being produced for each of these species across all sampling stream reaches and through time, as well as ensure continuity in the implementation of the survey. A series of objectives of the Río Yaqui fish stream monitoring program are identified. Additionally, many technological and statistical advances have resulted in improvements in data collection and analysis techniques since the depletion experiments began in 2004. We identified and implemented alternative approaches which were borrowed from the strengths of the traditional method used at San Bernardino and Leslie Canyon NWRs to help with standardization of methods used to survey these streams reaches, such as identifying steps to establish a closed population (to ensure that no individuals are immigrating/emigrating from the sampled area), identified a series of procedures to implement when starting and completing a depletion pass, and developed a statistical model to produce a "true" abundance estimate corrected for detection probability for all stream reaches and locations. Implementation of this protocol creates detailed documentation and standardization to ensure repeatability of future efforts. We sought to develop a data management system that could store the data online (Survey 123), on ServCat (USFWS Service Catalog), as well as be read into Program R to generate tables and figures for the annual and five year reports. In doing so, this protocol takes the existing approach and scientifically strengthens it by creating a series of steps that will allow the generation of results to be logistically easier and will help with reporting procedures.

Objectives

Management objectives (in priority order):

1) The Río Yaqui Fish Recovery plan identified criteria for downlisting based on whether these populations are secure now and also into the future (USFWS 1994). The primary objective of this protocol is to provide a robust means to quantify their current and future status.

2) Create spatially-explicit resource selection models to evaluate the effects of management activities and identify important habitat characteristics for the purpose of prioritizing other landscapes for re-introductions, as well as provide information to support restoration activities.

Sampling objectives (in priority order):

1) Provide an estimate of abundance of each species within the survey area with enough precision to detect a 5% population decline annually.

2) Create a spatially-explicit resource use model to predict abundance of Río Yaqui fish in relation to local stream characteristics (e.g., substrate, water temperature, wood debris, water flow, etc) for use in conservation planning efforts (i.e., habitat restoration, salvaging events, minimum flow criteria, and establishment of additional reintroduced populations for each species).

Element 2: Pilot Studies

Depletion sampling is a wide-spread sampling technique often used for estimating the abundance of demographically closed animal populations (Seber 1982). Depletion sampling is based on four primary assumptions (1) all animals have the same probability of capture, (2) the probability of capture does not change from one sample to the next (i.e., remains constant), (3) all removals from the population are known, and (4) the population is closed to any unknown changes (i.e., births, deaths, or migration) other than the known removals (Raleigh and Short 1981; Williams et al. 2002). Maintaining these assumptions and measuring the detection rate is problematic. For instance, assuming detection is constant over successive passes and the same for all animals and individuals is untenable. Heterogeneity arises from animal sex, age, size, individual variation, intensity of sampling, or sampling duration (Farnsworth et al. 2002; Peterson et al. 2004). Such heterogeneity is often ignored when modeling detection probability, resulting in biased estimates of abundance. This method requires repeated samples of a population in a specified area, on successive occasions, with animals captured and temporarily removed from the population (Williams et al. 2002). Fundamentally, the technique relies on a population diminishing in numbers as a fraction of the population is removed with each sampling occasion. The method estimates initial abundance, adjusted by a detection rate related to each sampling occasion, when multiple depletion passes are conducted (Dorazio et al. 2005). Therefore, efforts to test and pilot the technique were employed (see Stewart et al. 2019; Appendix B). These efforts provided useful insights for developing this protocol.

Simulation methods and analysis

We conducted extensive simulations to understand how true population size, declining detection probability over successive passes, model-based detection assumptions (using a constant or one of two variable detection functions), and the number of depletion passes affected bias of abundance (N) and detection probability (q). Please review Stewart et al. (2019) (i.e., Appendix B) for additional model-related specifications. To briefly describe the first simulation setup, we used all possible model combinations to evaluate how abundance and variable detection affected relative bias. We simulated five pass depletion counts from known abundances $N = \{20, 80, 100\}$ and detection probabilities $q = \{0.10,\ 0.20, \dots, 0.90\}$ using 1000 random draws from a binomial distribution. First, we assumed a constant detection rate across successive passes (e.g., five pass: $q\ =\ 0.10$ for all passes). Next, we assumed a 10%, 20%, and 30% decline in detection rate across successive passes from the initial first pass detection probability. To test the effects of variable detection while assuming constant detection, we fit the simulated data to the model using the parameterization of a constant detection function (e.g., $q_i \sim beta(a, b)$) across the 1000 trials. To test the effects of variable detection on models designed to account for variable detection, we assumed that detection declined monotonically from the initial pass (i.e., m1 = $\left(q_{ij} = p(1-p)^{j-1}\right)$, where $p = p_1 + (p_2 - p_1)\left(1 - c^{j-1}\right)$; Schnute 1983), and we also assumed that the rate of decline change to unknown magnitudes and specified a new model to account for the decline or increase in detection probability from the initial pass as m2 = $\left(q_{ij} = p_1(1-p_0)^{j-1}\right)$.

We completed a second simulation study using the very same models to examine if bias from variable detection across successive passes could be simply corrected by increasing the number of successive J passes (Bohrmann and Christman 2013). We simulated removal counts for $J =$

$\{3, 5, 8, 12\}$ pass depletion experiments having a known abundance (N = 100) and detection probabilities $q = \{0.10,\ 0.20, \ldots, 0.90\}$ using 1000 random draws from a binomial distribution. We first simulated the detection probabilities to remain constant across all passes. Next, we simulated three declines in detection rates of 10%, 20%, and 30% from the initial pass. To test the effects of variable detection while assuming using model-defined constant detection and variable detection functions, we fit the simulated data to the detection models described above.

Depletion models always provided unbiased estimates of abundance when detection probability was >0.60 (Figure 1 and 2). The size of the true abundance had greatest effect on the precision of the estimate. Smaller abundances generated more imprecise estimates. Reductions in detection probability resulted in greater bias in abundance. Estimates were biased most (-80% to -36%) when detection probability was <0.20. Each model was most biased when declines in fish detection between passes were highest (> 30% decline).

When the detection probability declined by 10% after each successive pass, and one assumes that detection probability remained constant (i.e., models the data using a constant detection function), then the estimated abundance was biased low, when detection was low <0.20. Otherwise, the results between models with varying detection (m1, m2) and constant detection are similar and produced unbiased estimates of abundance when detection >-0.20.

The second phase of the simulation explored tradeoffs in the number of depletion passes and fish detectability. Increasing the number of passes from 3 to 12 generally improved bias and reduced error when detection probability remained constant, or was 10% across successive passes (Figure 2). The number of successive passes did not improve bias when the decline in detection probability was >10% across successive passes.

When detection probability was constant, model m2 always produced unbiased estimates but with less precision when detection probability was <0.3. When detection probability declined by 10%, models produced unbiased estimates with detection was > 20%. When detection declined by 20 - 30% after each successive pass, unbiased estimates could be attained with detection probabilities >0.40 (for 20% decline between passes) and >0.60 (for 30% decline between passes).

Sample collection and field validation

We were also interested in understanding the significance of our simulations and how these transferred to “reality”. Therefore, we selected ten stream crossings located in and around San Bernardino and Leslie Canyon NWRs near Douglas, Arizona. These streams represent the extent of available habitats and also those located immediately downstream of known habitats having these species and represent the limited range of Yaqui chub, Yaqui topminnow, and Mexican longfin dace in the United States. At each of the ten stream reaches, crews established a 25 meter sampling unit, defined by two block nets (mesh size ≤ 3 mm) set upstream and the other downstream as a way to block off the sampling unit and establish a demographically closed population (i.e., prevent fish emigration and immigration). Block nets were visually inspected (i.e., feeling the bottom of the net) before, during, and after sampling to minimize fish escapement. At each stream section, experienced crews used a Smith-Root pulsed DC back pack electroshocking. Voltage and duty cycle of the back pack electroshocking was adjusted according to the conductivity of the stream and amperage output to approximate 0.10 continuous amperes and 1.0 peak amperes (Peterson et al. 2004). Crewmembers moved in an upstream direction, thoroughly sampled all available habitats (e.g., woody debris, undercut banks, pools, riffles), while maintaining a constant amount of effort among each of the five passes. During each successive pass, two biologists captured all individuals of each species encountered using dip nets, and captured fish were stored in aerated buckets identified by the electroshocking pass. Following fish sampling at each stream section, crews identified each fish to species, and then redistributed the individuals throughout the sampling unit once recovered (i.e., swimming and respiring normally).

In addition to sampling these ten stream crossings, we revisited three stream sections having only Yaqui topminnow. Here, we established three known populations (n = 20) of Yaqui chub to determine the validity and accuracy of depletion surveys to estimate abundance. Known populations were established by randomly dispersing a known number of Yaqui chub throughout a stream segment defined by an upstream and downstream block net, and these individuals were allowed to acclimate to their environment prior to sampling (Peterson et al. 2004). We sought to do the same for Yaqui topminnow as Yaqui chub but the availability of additional streams and permitting did not allow us to translocate the remaining two species to uninhabited stream reaches (i.e., translocating Yaqui topminnow from Leslie Creek to Turkey Creek).

Crewmembers measured water quality and physical habitat characteristics at each stream reach to provide stream reach-specific parameters for modeling detection probabilities and abundance of fish. Water quality data (water temperature (°C), specific conductivity (µs), and turbidity (NTU)) were recorded using calibrated meters before and after sampling. Following block net removal, a line-transect method was used to measure physical habitat characteristics. Transects were defined as being perpendicular to water flow, and started at the location of the lowermost block net and continued at 5-m intervals until crewmembers reached the uppermost block net. At each 5-m interval, crewmembers measured the streams wetted width. We recorded habitat characteristics such as substrate (classes: fine (< 6 mm), gravel (6-75 mm), cobble (75-150 mm), rubble (> 150 mm), stream segment type (pool, riffle, or run), and maximum depth (cm) at ¼ intervals across the transect (Peterson et al. 2004). Crewmembers visually quantified percent overhead cover (i.e., overhanding vegetation), turbulence (abrupt changes in water velocity), and undercut of the entire 25 m sampling unit. The contribution of wood was defined as the number of pieces within the stream channel measuring at-least 3 m long (Peterson et al. 2004).

Similar to the results from our simulation analyses, when detection probability was assumed to remain constant across successive passes, detection probability estimates were greater than estimates produced by the two variable detection models, regardless of species (Table 1). Both variable detection models produced abundance estimates more accurately than constant detection models. On average, mean abundance estimates were 63% and 74% higher for Mexican longfin dace, 66% and 116% higher for Yaqui chub, and 69% and 52% higher for Yaqui topminnow.

To assess accuracy of these models, we compared the model produced estimates to the known number of (n = 20) of Yaqui chub. Detection probability estimates produced by the constant detection probability model ranged from 0.28 to 0.47 and estimated stream reach-specific abundance ranged from 11 to 13. The posterior probabilities of the 95% CI's did not overlap with the known population size of 20 (Table 1). This bias in abundance estimates indicates that detection probability varied across successive passes. Detection probabilities for models that allowed variability across successive passes ranged from 0.18 to 0.37 and 0.26 to 0.45. Estimates for stream reach-specific abundance ranged from 19 to 20 and 15 to 16, and CIs overlapped with the known population size. Though both variable detection models performed similarly, we used the variable detection model $p = p_1 + (p_2 - p_1)(1 - c^{j-1})$ for all subsequent analyses (m1). We based this selection given that it produced higher precision in parameter estimates, the mean posterior probabilities of stream reach-specific abundance (19 to 20) were closer to "truth" when compared to the known Yaqui chub populations, and the model attained convergence with fewer iterations (Table 1).

The model structure containing the population-level random effect performed better than those models that were formulated without the population-level random effect. Posterior probabilities for inclusion parameters of stream reach-specific environmental correlates confirmed that detection probability and abundance were affected by habitat factors. For all three species, a significant influence of stream area and substrate composition was indicated (Table 2). Abundance of Yaqui chub and Yaqui topminnow increased with stream area, but as stream area rose, then abundance of Mexican longfin dace decreased. Abundance of Yaqui chub increased and abundance of Yaqui topminnow decreased with increasing channel width. Abundance increased by 90% when channel width increased from 220 cm to 360 cm; while abundance of Yaqui topminnow decreased by 65%. A significant influence of stream depth was also indicated for abundance of Yaqui chub (Pr = 0.95). As stream depth increased from 15 cm to 30 cm abundance of Yaqui chub increased by 78%. Abundance of Yaqui topminnow increased in pool habitats. Increases in fine sediment were associated with a decline in abundance for Mexican longfin dace.

The estimated detection probabilities for Yaqui topminnow at a stream depth of 30 cm was 5% (95% CI = 2%, 10%; Table 2). Mexican longfin dace, detectability decreased as percent gravel substrate rose. Channel unit increases from riffle to pool habitats associated with declines in detection for Yaqui chub. The estimated detection probability for Yaqui chub in riffles and pools was 40% (95% CI = 34%, 44%) and 22% (95% CI = 18%, 26%). Detection probability increased with increased stream flows, with the estimated detection probability at 0.00 and 0.50 was 18% (95% CI = 14%, 22%) and 48% (95% CI = 43%, 53%).

Lessons learned from pilot studies

Our results provided useful insights for improving Río Yaqui fish monitoring efforts:

1. Abundance estimates were negatively biased when detection probability declined by 20% and 30% across successive passes, or if detection probability was <0.20. To reduce the extent of bias contributed to declining detection probability, one must maintain a closed population, reduce the duration of the survey, use identical collection methods, and standardize effort during each removal (Raleigh and Short 1981).

2. Abundance estimates remained negatively biased for increased population sizes in our simulations (Figure 1). Increasing the number of depletion passes per stream reach improved precision of the abundance estimates but failed to improve bias (Figure 2).

Therefore, when detection probability is low and variable, bias is not corrected by increasing sampling effort. These results add to the message that sampling without block nets, variable effort, and not accounting for variables affecting detection probability (such as habitat) will increase bias regardless of the number of passes used to survey a stream reach.

3. Simulations identified that estimating abundance using a depletion experiment can be a reliable monitoring method as long as the variability in detection probability across successive passes is less than 10% or if the detection probability is >0.60. This requires implementing the survey correctly so that any violations in survey assumptions are mitigated.

4. A likely cause of variable detection encountered historically was due to unstandardized effort during each removal. Since depletion sampling requires constant effort, a watch or timer worn by a crewmember could be a viable method to improve standardization. Furthermore, our assessment also identified that more fish were captured and the decline in numbers captured across successive passes became more prominent when sampling for ~300 seconds. It is our recommendation that members of the survey crew sample at a rate of ~300 seconds per pass at each stream location.

5. A set of circumstances was identified for when depletion surveys work and do not work. Consequently, we invested in significant pilot work and established known populations to quantify the extent of bias in the field. Our estimates produced by both variable detection models (see m1 and m2 above) for stream reach-specific abundance ranged from 19 to 20 and 15 to 16, and CIs overlapped with the known population size (Table 1). Though both variable detection models performed similarly, we recommend using the variable detection model $p = p_1 + (p_2 - p_1)\left(1 - c^{j-1}\right)$ for all subsequent analyses (m1) because it produced more precise estimates, estimated abundances (19 to 20) were closer to truth, and models attained stationarity faster than m2.

6. Detection probabilities varied considerably by species and stream reach (Table 2). For example, we estimated that Yaqui chub were more difficult to capture in pools and in streams having high percent of undercut bank, whereas Yaqui topminnow were more difficult to capture when sampling gravel substrate streams and in deeper water. These outcomes are likely due to gavel streams having greater areas for concealment and also being associated with faster flowing stream reaches that decreased capture efficiency. Decreased netting effectiveness in deep water likely prevented effective capture of Yaqui topminnow. Regardless, habitat was a significant source of the variation in detection probability for each species. Therefore, it is critical to measure habitat in the field to later account for this extra-variation in the depletion model as a method to produce unbiased estimates of abundance.

7. Because the model-based estimates for the pilot work produced unbiased estimates of the known population (Table 1), we were able to identify a set of rules to quantify how many successive passes should be made at a stream reach. We recommend sampling for a minimum of 5 successive passes, and then sampling until one captures 0 or 1 fish for a minimum of two additional passes. However, if zero fish are captured on the fourth and

fifth pass, then sampling ceases. If more than one fish is captured on the 5th pass, then at-least two additional successive passes are needed. The reason behind this approach ensures an observed decline and that the majority of the fish are removed from the stream reach. This is important because the infinite summation of the integrated likelihood for the abundance model is replaced by the summation of observations (Dorazio et al. 2005). This ensures precision and accurate estimation of the detection probability.

8. The statistical approach used by the former survey assumed that catch declined linearly, did not account for variable detection, and was used to calculate an abundance and detection probability estimate for each stream reach and year separately for each surveyed species. Trend information in abundance was not considered in the model. Therefore, we present a novel modification of a hierarchical Bayesian mixture depletion model, where our model leverages information from multiple stream reaches and years as a means to improve precision of the estimator by assuming that population-specific parameters are derived from population distributions.

Table 1. Number captured, and mean detection probabilities (p; 95% CI) and stream reach(site)-specific abundance (N; 95% CI) of Mexican longfin dace (*Agosia sp.*), Yaqui chub (*Gila purpurea*), and Yaqui topminnow (*Poeciliopsis occidentalis sonoriensis*) using three different detection probability models.

			Detection function					
			$q \sim Beta(b,c)$		$q = p(1-p)^{j-1}$		$q = p_1(1-p_0)^{j-1}$	
Species	Stream sites	Catch	p	N	p	N	p	N
Mexican longfin dace	Leslie Creek Site 1	2	0.16 (0.09, 0.24)	4 (2, 9)	0.09 (0.05, 0.14)	7 (2, 18)	0.11 (0.02, 0.22)	13 (12, 17)
	Minckley Site	12	0.65 (0.45, 0.82)	12 (12, 13)	0.47 (0.28, 0.65)	18 (13, 25)	0.64 (0.39, 0.82)	17 (12, 24)
	Turkey Creek Site 1	85	0.35 (0.31, 0.40)	96 (89, 106)	0.22 (0.19, 0.25)	158 (135, 186)	0.28 (0.16, 0.37)	128 (96, 212)
	Turkey Creek Site 2	420	0.23 (0.18, 0.29)	576 (512, 664)	0.14 (0.11, 0.17)	988 (853, 1168)	0.15 (0.06, 0.24)	1032 (582, 2357)
	Turkey Creek Site 3	324	0.41 (0.37, 0.45)	349 (337, 364)	0.27 (0.24, 0.29)	557 (514, 604)	0.28 (0.25, 0.43)	535 (453, 665)
	Turkey Creek Site 4	241	0.46 (0.40, 0.51)	253 (245, 264)	0.31 (0.27, 0.36)	393 (359, 431)	0.43 (0.34, 0.51)	282 (252, 334)
Yaqui chub	Leslie Creek Site 1	9	0.03 (0.01, 0.51)	75 (26, 140)	0.02 (0.01, 0.05)	136 (50, 296)	0.02 (0.01, 0.06)	235 (48, 1030)
	Leslie Creek Site 2	4	0.36 (0.23, 0.48)	5 (4, 7)	0.21 (0.12, 0.30)	9 (4, 16)	0.19 (0.04, 0.40)	12 (4, 38)
	Turkey Creek Site 2	3	0.20 (0.07, 0.38)	5 (3, 12)	0.13 (0.04, 0.25)	8 (3, 21)	0.15 (0.03, 0.34)	11 (3, 43)
	Turkey Creek Site 5	198	0.37 (0.30, 0.44)	221 (207, 241)	0.24 (0.18, 0.30)	356 (313, 412)	0.21 (0.02, 0.40)	708 (254, 3476)
	Turkey Creek Site 6	58	0.44 (0.30, 0.55)	62 (58, 72)	0.26 (0.16, 0.36)	102 (82, 131)	0.23 (0.05, 0.48)	157 (75, 493)
	Twin Site	10	0.28 (0.09, 0.51)	13 (10, 22)	0.19 (0.06, 0.38)	21 (12, 37)	0.20 (0.04, 0.47)	26 (10, 75)

	Known site 1 ($n = 20$)	10	0.28 (0.09, 0.51)	12 (10, 19)	0.18 (0.06, 0.35)	19 (12, 31)	0.26 (0.13, 0.50)	16 (10, 32)
	Known site 2 ($n = 20$)	11	0.53 (0.27, 0.75)	11 (11, 13)	0.37 (0.16, 0.58)	20 (14, 26)	0.45 (0.15, 0.71)	15 (14, 31)
	Known site 3 ($n = 20$)	12	0.47 (0.23, 0.69)	13 (12, 15)	0.31 (0.13, 0.50)	19 (14, 27)	0.40 (0.15, 0.67)	16 (12, 33)
Yaqui topminnow	Leslie Creek Site 1	14	0.05 (0.01, 0.26)	253 (17, 1104)	0.02 (0.01, 0.09)	507 (42, 3164)	0.04 (0.01, 0.15)	213 (33, 849)
	Leslie Creek Site 2	73	0.69 (0.59, 0.78)	73 (73, 75)	0.50 (0.40, 0.59)	104 (91, 119)	0.68 (0.56, 0.79)	76 (73, 84)
	Minckley Site	94	0.29 (0.19, 0.39)	118 (100, 149)	0.16 (0.09, 0.24)	208 (158, 302)	0.15 (0.02, 0.32)	350 (152, 1400)
	Twin Site	807	0.45 (0.42, 0.48)	850 (833, 870)	0.30 (0.28, 0.33)	1325 (1258, 1398)	0.39 (0.28, 0.46)	1041 (874, 1417)

Table 2. Estimated detection probability and parameters for Mexican longfin dace (*Agosia sp.*), Yaqui chub (*Gila purpurea*), and Yaqui topminnow (*Poeciliopsis occidentalis sonoriensis*). SD = Standard Deviation. Pr = Inclusion probability. CI = Credibility Intervals.

Parameter	Mean slope (SD)	95% CI	*Pr*	Bayesian *p*-value (SD)	95% CI
Mexican longfin dace					
Abundance					
Area	-0.45 (0.17)	-0.76, -0.141	0.79	38 (3.71)	33, 47
Gravel	0.40 (0.18)	0.12, 0.81	0.77		
Fine	-1.71 (0.29)	-2.30, -1.13	0.82		
Detection probability					
Gravel	-0.46 (0.08)	-0.63, -0.31	0.95		
Yaqui chub					
Abundance					
Stream depth	1.02 (0.26)	0.90, 1.31	0.95	32 (4.32)	26, 42
Channel width	0.86 (0.25)	0.52, 1.42	0.80		
Area	0.57 (0.31)	0.12, 1.25	0.83		
Undercut	0.97 (0.50)	-0.11, 1.82	0.73		
Detection probability					
Channel unit	-0.62 (0.09)	-0.85, -0.50	0.75		
Stream flow	1.23 (0.22)	0.80, 1.65	0.72		
Undercut	-0.13 (0.54)	-1.26, 0.82	0.71		
Yaqui topminnow					
Abundance					
Channel width	-2.75 (1.46)	-6.33, -1.05	0.71	64 (3.50)	57, 71
Channel unit	0.77 (0.70)	0.10, 2.75	0.95		
Area	2.18 (1.12)	0.29, 3.92	0.70		
Stream flow	-3.08 (1.59)	-6.56, -1.07	0.75		
Detection probability					
Stream depth	-1.02 (0.02)	-1.07, -1.01	0.71		

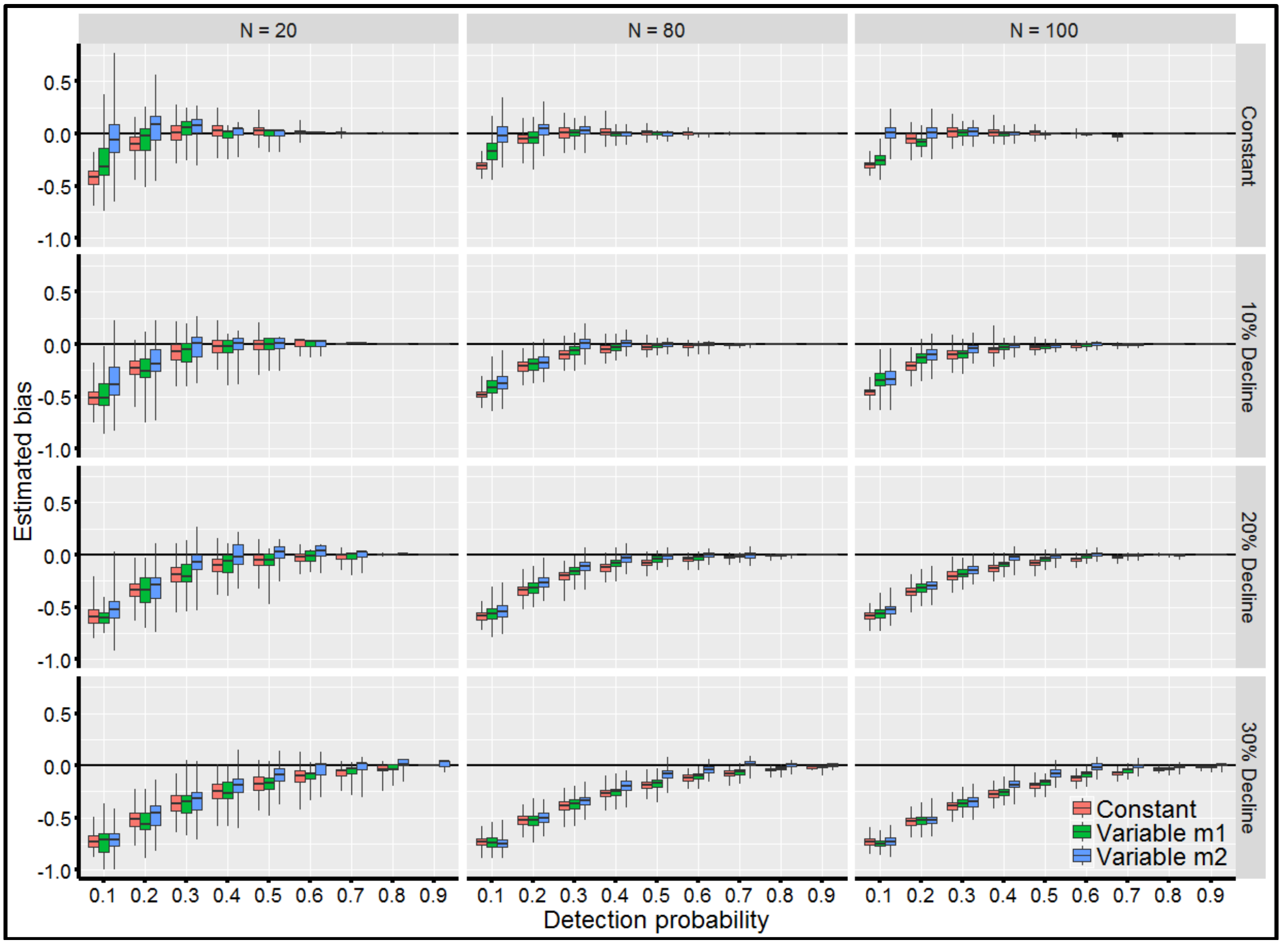

Figure 1. Estimated bias in detection probability using 5-pass depletion sampling at three different abundance (20, 80, 100) levels with no decline (constant), 10% decline, 20% decline, and a 30% decline in detection probability across five successive passes. Results shown are from the three different detection models (Constant $q \sim Beta(a, b)$, Variable m1 $p = p_1 + (p_2 - p_1)(1 - c^{j-1})$, Variable m2 $q = p_1(1 - p_0)^{j-1}$).

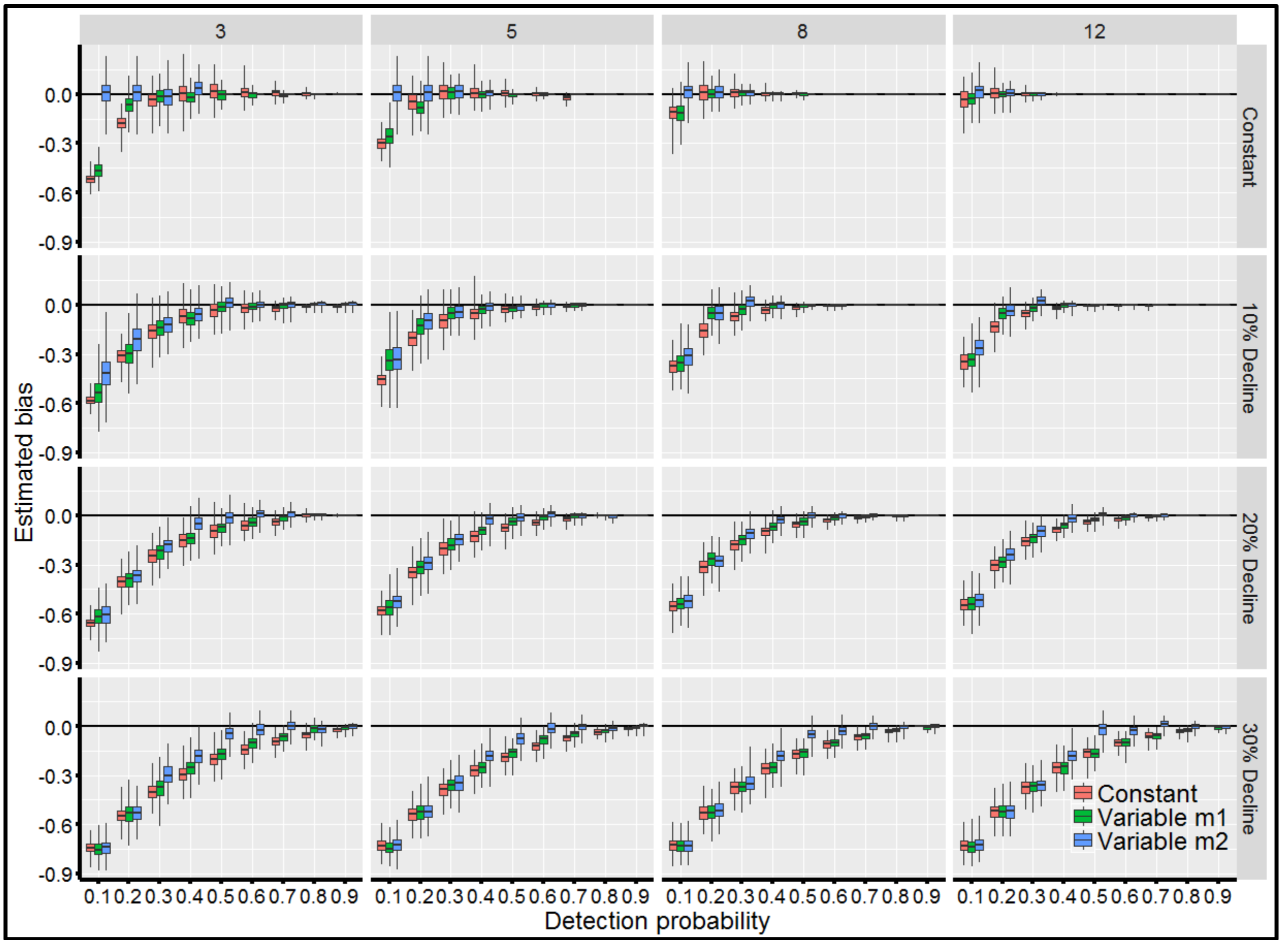

Figure 2. Estimated bias in detection probability using 3, 5, 8, and 12-pass depletion sampling with no decline (constant), 10% decline, 20% decline, and a 30% decline in detection probability across successive passes. Abundance was defined as 100 and results shown are from the three different detection models (Constant $q \sim Beta(a, b)$, Variable m1 $p = p_1 + (p_2 - p_1)(1 - c^{j-1})$, Variable m2 $q = p_1(1 - p_0)^{j-1}$).

Element 3: Sampling Design

Sample design

This protocol is designed to provide methodology for monitoring status and trends in fish (Yaqui topminnow, Yaqui chub, and Mexican longfin dace) abundance in streams at and around San Bernardino and Leslie Canyon NWR's and El Coronado Ranch (West Turkey Creek, AZ). Secondarily, the protocol provides a mechanism to quantify important species-habitat relationships, which can help quantify a species life-history requirements and consequently identify which of these factors affect their conservation status and trends through time. Finally, this information in the future can be used to identify other potential suitable stream reaches for reintroductions to continue expanding the range of each of these species by establishing new subpopulations in suitable habitats.

Target universe

The biological population in which inference is intended is a subset of the Río Yaqui fish species and their populations (i.e., Yaqui chub, and Yaqui topminnow) and also the Mexican longfin dace that inhabit streams located in the United States (San Bernardino and Leslie Canyon NWR's, and El Coronado Ranch (West Turkey Creek, AZ)). However, the stream geometry and depth of Black Draw at Leslie Canyon NWR may place logistic constraints on monitoring these fish populations during wet years, where the increased depths present a potential electrical hazard to staff wading in this stream while equipped with a backpack electroshocking unit. Therefore, we focus monitoring efforts only on those populations found in streams at San Bernardino NWR and El Coronado Ranch (West Turkey Creek, AZ), which comprises greater extent of the distribution of these species in the United States. During low flow years at Black Draw at Leslie Canyon NWR, this protocol can be used to draw inference to the status of these populations. This protocol can also be applied to other nearby streams in the event that new Río Yaqui fish populations are created through translocation. Currently, populations are surveyed annually at several stream reaches where these species are known to occur and also those located downstream to evaluate population expansion.

Sample frame and sampling units

The sampling frame consists of those streams (not the stream reach) where Río Yaqui fish are known to occur and are found within the two primary sampling units (San Bernardino NWR and El Coronado Ranch (West Turkey Creek, AZ)). These units are geographically distinct; therefore the sampling frame by design is a hierarchically nested sampling system. The sampling system includes 3 hierarchical levels: unit (San Bernardino NWR and El Coronado Ranch), stream (those streams located within these units), and stream reach (also known as the site). The highest level is the two primary sampling units (defined above). In each of these units, one to three streams are nested; in each stream, a handful of stream reaches are known to house isolated populations of Río Yaqui fish and we also sample those stream reaches located downstream of these areas to evaluate population expansion. At each of these stream reaches, a depletion experiment will be used to estimate true abundance and species-habitat relationships. It is well documented through our historical assessments that Río Yaqui fish are not found outside of these known isolated populations. Our estimates of annual abundance will apply to each stream reach.

Sample selection and size
Ten stream reaches within the two sampling units will be sampled annually. These species are found in these select areas and nowhere else in southeastern Arizona. For example, depending on the availability of water, approximately three to four stream reaches (Leslie Creek (n = 2; Site 1 = UTM(12) 0641482, 3495950; Site 2 = UTM(12) 064163, 3496076), Minckley (n = 1; UTM(12) 0665030, 3468928), and Twin Site (n = 1; UTM(12) 0665172, 3468611)) are available to sample at San Bernardino NWR. Moreover, at El Coronado Ranch (West Turkey Creek, AZ), though water does not necessarily pose an issue, approximately two to three streams house Río Yaqui fish species. Since their translocation in the early 2000s, Yaqui chub populations have not expanded due to the lack of suitable habitat found throughout the remaining parts of the stream at West Turkey Creek, AZ. At-least two to three known locations at West Turkey Creek, AZ are known to house self-sustaining populations. Therefore, three stream reaches and three located 100 m immediately downstream of them at West Turkey Creek, AZ will be surveyed annually using this protocol. Their coordinates are: Site 1 = UTM(12) 0651053, 3526946; Site 2 = UTM(12) 0651034, 3526946; Site 3 = UTM(12) 0650992, 3526429; Site 4 = UTM(12) 0650877, 3526951; Site 5 = UTM(12) 652787, 3526648; Site 6 = UTM(12) 652810, 3526663). Therefore, for analysis of population trend, the estimates of annual abundance will apply to a specific stream reach identified by their Easting and Northing coordinates above and again in SOP 1. The stream reaches will be set annually and will be surveyed near and around these coordinates, given that water may pose an issue at the exact location. In the event that the distribution of Río Yaqui fish sub-populations increase in the future, then the number of stream reaches sampled annually in each sampling unit will increase with each new population and an associated stream reach located 100 m immediately downstream of this new population to monitor population expansion.

Survey timing and schedule
Observers should conduct surveys annually in September-October and when detection probability is highest and least affected by habitat variables that reduce detection probability (e.g., increased water depths and stream flows). Therefore, this estimate will produce an annual fall/winter Río Yaqui fish abundance estimate. Specific guidance on how to conduct the depletion experiment is included in Element 4 and SOP 1.

Note: *Now that we are accounting for how habitat affects detection probability and local abundance, and how this varies across space and time in the survey protocol and model-based assessment, it is no longer required that all stream reaches must be surveyed the same week.*

Sources of error
Not using block nets appropriately will bias estimates of abundance. To maintain population closure, nets (mesh size < 3 mm) set to define the upstream and downstream boundaries of the 25 m stream reach is required to establish a population to survey, regardless of whether a stream is located at San Bernardino NWR or El Coronado Ranch (West Turkey Creek, AZ). During sampling nets may be affected by environmental conditions. This increases the likelihood of bias because fish may leave the stream reach by swimming around, under, or even over the net. Prior to the survey, nets must be inspected to ensure that each net extends vertically through the water column and horizontally to both sides of the stream bank. The top of each block net must be at a minimum 12 inches above the water surface. Following a depletion pass, nets must be inspected to ensure that fish are not able to escape.

To account for non-constant imperfect detection, the number of seconds per pass must remain constant throughout the depletion experiment. It is important that each pass has similar effort so that any variation in catch per pass is a result of the sampling process. Therefore, we suggest that a timer (e.g., stopwatch) be used during sampling to assist with monitoring the time that a user spends sampling each pass, with the ultimate goal of maintaining approximately the same amount of effort each pass (~300 “on-time” seconds).

The number of passes will no longer remain fixed to three but will increase to a minimum of 5 passes per stream reach, though it is likely that on some occasions the number of passes will be greater than 5. To explain how, users will sample the minimum of 5 depletion passes required for each stream reach, but after 5 passes, sampling will continue until at-least a 0 or 1 count of each species is counted on two successive passes. If a 0 or 1 count is observed on the 4th pass and also the 5th pass, then sampling ceases. If a 0 or 1 count is observed on the 5th and also the 6th pass, then sampling ceases. However, if a 0 or 1 count is not observed for at-least two passes by the 5th pass, then sampling continues for at-least two additional passes to ensure that an adequate decline in the number of individuals captured per species was attained. No more than 10 passes will be completed per stream reach.

Element 4: Field Methods and Processing of Collected Materials

Pre-survey logistics and preparation

Data collection requires one team leader and at-least one other experienced member, as well as all personal protective equipment necessary for backpack electroshocking. The team leader should be trained in electroshocking safety, theory, and operation. At-least two crewmembers on an electroshocking team must have a current certificate in CPR and First Aid Training. If possible, and in the event of three crewmembers, one of the two trained crewmembers must be stationed on the bank during the survey. Before each survey, the team leader should discuss the tasks and procedures of wading and working in streams, any and all hazards (slips, falls, sprains, eye injuries, drowning, fatigue, exposure, and electrocution), abatement actions so that employees have read and understand the contents and expectations, and ensure that all individuals are qualified to perform the work project or activity. Backpack electroshocking should not be attempted if the average depth of water is too deep for operators to wade at less than “thigh depth” for the majority of the exercise (e.g., Black Draw at Leslie Canyon NWR). Suspend wading operations if adverse weather or water conditions are a safety concern (i.e., thunder, lightning, swift water/ extreme flow conditions). Lastly, do not enter the water if you are unable to swim or are uncomfortable with your swimming abilities.

One member of the survey crew will collect data on a tablet computer or datasheets. Data collected will include the number of individuals captured per species and depletion pass, the spatial location of each stream reach, the length of a stream reach, number of netters used during sampling, water quality data (water temperature (°C), pH, specific conductivity (µs), dissolved oxygen, turbidity (NTU), and total algal), and physical habitat characteristics (wetted width (cm), substrate characteristics, stream segment type (pool, riffle, or run), and maximum depth (cm)). The techniques used to collect this data will be described in more detail below. Equipment should be properly calibrated and vetted prior to its application to ensure ease of use, data integrity, and security. Please refer to the gear checklist (Table 3). The use of trade, firm, or product names is for reference only and does not constitute endorsement of any nature.

Personal protective equipment

The personal protective equipment needed when backpack electroshocking is stream dependent but generally includes (Department of the Interior 2016):

1. Properly fitting, sturdy boots with non-slip soles and adequate ankle support should be worn at all times while wading.
2. Waders will be worn when wading in streams to reduce the risk of exposure to cold water temperatures.
3. A wading belt should be worn when wearing chest waders.
4. Personal floatation devices should be used if water conditions are greater than “knee depth.”
5. Polarized glasses should be worn to protect eyes from hazards and to reduce glare from the water surface, which improves stream bottom visibility.
6. Wear weather appropriate clothing and be prepared for adverse conditions (i.e., rainwear).
7. Use sunscreen to reduce damage from sun exposure.

Backpack electroshocking policy and guidance:
Consult the following policy and guidance when planning backpack electroshocking activities:

1. U.S. Fish and Wildlife Service requirements for electroshocking safety (241 FW 6; https://www.fws.gov/policy/241fw6.html#section_6_4).
2. Basic Program Elements for Federal Employee Occupational Safety and Health Programs and Related Matters (29 CFR 1960).
3. Executive Order 12196, Occupational Safety and Health Programs for Federal Employees.
4. Federal Agency Safety Programs and Responsibilities (P.L. 91-596, Section 19).
5. National Fire Protection Association (NFPA) 70, National Electric Code, Current Edition.
6. Occupational Safety and Health Administration (OSHA), Occupational Safety and Health Standards (29 CFR 1910).
7. 485 Department Manual (DM) 22, Watercraft Safety.

Table 3. Equipment checklist for backpack electroshocking.

Gear	Comments
Survey Protocol Framework for Monitoring Abundance of Río Yaqui Fishes in Streams	
Copies of datasheets and previous datasheets for the stream reach	Previous datasheets will assist with standardizing effort with previous events.
Pencils	
GPS	
Watch or other timepiece	
Water quality field meter	To record conductivity and water temperature
Measuring tape	For measuring reach lengths and habitat data Use long measuring tapes (25-100 m)
Small aquarium net	For retrieving fish from buckets for enumerating
Aerator (battery powered)	Requirement to reduce stress of fish One per bucket recommended (10 buckets total)
Backpack Electroshocking Machine (BEM)	
Two block nets	Mesh size <3 mm or 1/8”
Dip nets	Mesh size <3 mm or 1/8”
BEM batteries	Including spare batteries
Electrical tape	For protecting battery terminals
Heavy-duty rubber gloves	
Waders	One pair for each team member
Wader repair kit	
Personal flotation device	Optional unless stream is greater than “knee depth”
Polarized sunglasses and hat	Set for each team member
Long-handled dip nets with insulated handles	
Buckets with tight fitting lids for holding fish	Recommend a minimum of 10 buckets to ensure a bucket for each depletion pass.
Fiberglass stream gauge	

Flow meter to characterize stream velocity	Recommend either FlowTracker, Marsh-McBirney, or Global Water Flow Probes
Flagging tape/ permanent markers	For marking out and labelling subreaches
Day pack w/drinking water and food	

Establishment sampling units

Stream reaches are predetermined. They included those where Río Yaqui fish species are known to occur and also those located downstream within the San Bernardino NWR and El Coronado Ranch (West Turkey Creek, AZ) sampling units. These predetermined sampling units are intended to remain static across surveys and years: Leslie Creek (n = 2; Site 1 = UTM(12) 0641482, 3495950; Site 2 = UTM(12) 064163, 3496076); Minckley (n = 1; Site 3 = UTM(12) 0665030, 3468928); Twin Site (n = 1; Site 4 = UTM(12) 0665172, 3468611); Site 5 = UTM(12) 0651053, 3526946; Site 6 = UTM(12) 0651034, 3526946; Site 7 = UTM(12) 0650992, 3526429; Site 8 = UTM(12) 0650877, 3526951; Site 9 = UTM(12) 652787, 3526648; Site 10 = UTM(12) 652810, 3526663). Therefore, stream reaches within each sampling unit do not require selection on an annual or survey-specific basis. This, of-course, depends on whether or not in the future if new populations are successfully established. In the event of ample water, all pre-determined stream reaches will be surveyed annually. Stream reaches within each sampling unit do not have to be surveyed chronologically in ascending or descending order. They can be sampled in an order that is most efficient, safe, and logistically beneficial.

Data collection procedures (field, lab)

We focus on depletion (i.e., removal) sampling, often used for estimating the abundance of demographically closed animal populations (Seber 1982). This method requires repeated samples of a population in a specified area, on successive occasions, with animals captured and temporarily removed from the population (Williams et al. 2002). Fundamentally, the technique relies on a population diminishing in numbers as a fraction of the population removed with each sampling occasion. The method estimates initial abundance, adjusted by a detection rate related to each sampling occasion, when multiple depletion passes are conducted (Dorzaio et al. 2005).

Depletion sampling has four assumptions: (1) all animals have the same probability of capture, (2) the probability of capture does not change from one sample to the next (i.e., remains constant), (3) all removals from the population are known, and (4) the population is closed to any unknown changes (i.e., births, deaths, or migration) other than the known removals (Raleigh and Short 1981; Williams et al. 2002). Maintaining closed populations, using identical collection methods, and standardizing effort during each removal step, are useful methods and imperative for maintaining sampling assumptions (Raleigh and Short 1981).

Establish sample reach

At each predetermined stream reach, crews will establish a 25 meter sampling reach by staying out of the water and measuring the length of the stream reach from the stream bank. Select and mark off the sample reach start and end points with flagging tape. Start and end points are defined so that block nets (mesh size ≤ 3 mm) can be stretched across the upstream and another at the downstream location as a way to block off the sampling unit and establish a demographically closed population (i.e., prevent fish emigration and immigration). Block nets must be visually inspected before sampling and before each sampling pass to minimize fish escapement, such that no space exists between the streambank and net, the net is stretched from

12 inches above the surface of the water to the stream bottom. Nets should be inspected on the side of the net that does not occur within the stream reach being sampled to minimize disturbance to the surveyed area. It is recommended that you weight the bottom net using a rock to ensure that the net stretches from the substrate to 12 inches above the water surface.

Record GPS coordinates of the sampling unit

Use the GPS (e.g., Garmin) to determine the Easting, Northing, and the UTM Zone at the downstream block net. Record the GPS reading, along with stream name, stream reach ID (from previously prepared sampling unit list), date, and crewmember names on the data sheet.

Water quality measurements

Prior to fish sampling, water quality characteristics will be measured using **calibrated** meters at each study reach downstream from the lower block net for each sampling occasion. Whether it is an YSI meter or a meter of choice, annual calibration practices should be implemented prior to field sampling as per any and all guidance issued in the user manual of each meter. Meters should be checked for accuracy against standard priors to sampling each day. Water quality should be collected using these meters downstream of the downstream block net. First, lower the probe into the stream, and then allow the probe to equilibrate. When field measures no longer appear to be changing, record water temperature to the nearest 0.1 degree Celsius (°C), pH to a value of 0.1, specific conductance to 0.01 microsiemens per centimeter (µs/cm), dissolved oxygen (DO) to the nearest 0.01 milligram per liter (mg/L), turbidity (NTU), and total algal records on the printed data sheet or in the tablet computer.

Calibrating the electroshocking unit

The instructions to calibrate an electroshocking unit are identified for the Smith-Root backpack electroshocking device. Make sure prior to assembly that the electroshocking unit is turned to the off position. The unit is off when the red knob on the top is rotated completely counter clockwise. Assemble the electroshocking unit by first securely placing a freshly charged battery within the backpack unit. Properly attach the anode (pole with aluminum ring) and the cathode (black rat tail) to their respective output connectors on the bottom rear of the instrument case of the electroshocking unit, and then replace the battery compartment cover and re-secure the latches. A crewmember should then help place the backpack on the individual who will be electroshocking. Turn on the unit. The unit, voltage and duty cycle, should be adjusted based on the environmental conditions measured at the stream reach prior to sampling. It is important to test each setting to ensure a nonlethal fish response. Specific guidance on how to adjust the backpack electroshocking unit settings is included in SOP 1. These settings should be recorded for each stream reach in the datasheets or tablet computer.

Depletion sampling

A minimum of 10 buckets filled with stream water from a location below the downstream block net prior to beginning sampling should be placed on the stream bank, equipped with their own aerator, and distinguished by the specific depletion pass number. Crewmembers should either label the side of each bucket with a specific number (1-10) or use uniquely identifiable flagging tape to mark their handle.

Prior to starting the first pass, zero the time on the left side of the electroshocking unit, as well as equip a person (i.e., preferably a person on the bank) with a timing device to help monitor the number of seconds per pass during sampling. In the pilot study, on average a single electroshocking pass was approximately **<u>300 “on-time” seconds in duration,</u>** which is the amount of “on-time” seconds that it takes to move through the stream reach, and more than adequate to estimate “true” abundance of Río Yaqui fishes (Stewart et al. 2019). We recommend using a stopwatch or any other timing device to accompany sampling because it is difficult to keep track of time when the number of “on-time” seconds is located on the back of the electroshocking unit. Keeping a relative track of time is important because some passes may receive greater effort than others depending on the environmental conditions, species, and the numbers of fish encountered. For example, when a lot of fish are encountered, it is easier for crewmembers to methodically collect fish and move upstream. This can take considerably more time in comparison to those passes where few fish are captured, or when one implements the final pass of the survey during a long day. Therefore, and to help standardize effort among passes, it is also beneficial for either a crewmember located on shore or equip the netter with a timing device on the inside of their wrist and in view as a method to keep pace. In doing so, one should have a relative idea of about how long it may take to complete the 300 “on-time” seconds for each pass. This is being implemented to control for survey effort, ensure consistent effort is being applied across all passes, and will help mitigate some of the error associated with heterogeneity in detection probability that we now know can bias the model-based information being produced from depletion assessments (Stewart et al. 2019).

Crewmembers should enter the stream reach at the downstream block net. Electroshocking should proceed upstream with bank-to-bank sweeping of the anode pole. This sweeping technique is used to ensure maximum coverage of the area being sampled and that all available habitats are properly targeted. Netters should net all stunned fish and place them into one of the uniquely identifiable buckets used for the specific pass until the entire reach has been sampled.

Once the crew reaches the upstream block net, the anode should be run along the entire length of the block net using a wafting technique to draw stunned fish away from the net and pulled towards the crew. The netter should sweep the net several times to ensure that all captured fish that retreated to the net have been removed.

The timer on the personal watch (e.g., stop watch) should be stopped. This will give an index of how long it takes to actually complete 300 “on-time” seconds, and also ensures that some passes are not receiving greater effort than other passes at the time of the survey. Afterward, the uniquely identifiable bucket (designated for the specific depletion pass: 1-10) containing stunned fish should be placed securely on the shoreline, the aerator turned on, and fish visually inspected to ensure quick recovery (If fish do not recover quickly, then the settings (voltage, pulse width, and pulse rate) should be decreased). Then, record the number of seconds per pass as identified on the electroshocking unit either on the data sheet or in the table computer. Zero the time on the personal watch and electroshocking unit.

Next, both block nets should be visually inspected after each pass to ensure they remain stretched from bank-to-bank and stretched from 12 inches above the water surface to substrate. If at any time the block nets are washed downstream, then sampling should be discontinued for the day, fish should be returned to the stream reach, and the stream reach should be re-surveyed exactly one week later. If after the second or third pass of sampling the integrity of the block nets change and are no longer stretching from bank to bank, then the surveyors should discontinue sampling, repair the nets, and then continue sampling the stream reach. The netter should then retrieve the bucket required for the next pass, wade into the stream reach, start the timer on the personal watch to keep track of pace, and begin collecting stunned fish. The member with the electroshocking unit is to continue the bank-to-bank sweeping of the anode pole in an upstream direction. A minimum of 5 successive passes will be completed per stream reach. Thereafter, sampling will continue until a zero or a count of one individual is observed on at-least two successive passes after the initial 5. For example, sampling will conclude if a zero or a count of one is observed on passes 6 and 7. However, if a count >1 is observed on either pass 6 or 7, then the crew will continue sampling for an additional pass. If during this additional pass (say passes 7 and 8 or passes 8 and 9) a count of a zero or one is not observed on two consecutive passes, then repeat the steps above. If a zero or one is observed on two successive passes, such as pass 5 and 6, or any combination after (such as 7 and 8), then sampling of the stream reach is completed. After then 10th depletion pass, then sampling ceases. Please remember to keep notice of the number of seconds per pass and visually inspect each block net before and after each depletion pass.

Environmental covariates
Immediately after depletion sampling, physical in-stream habitat characteristics will be measured using a line-transect method by establishing transects perpendicular to flow every 5 m starting from the downstream block net (transect 1) toward the upstream block net (transect 6) of the 25 m stream reach (Figure 3). Transects 1 through 6 are identified for each habitat variable being measured for each stream reach on the data sheet (Appendix C).

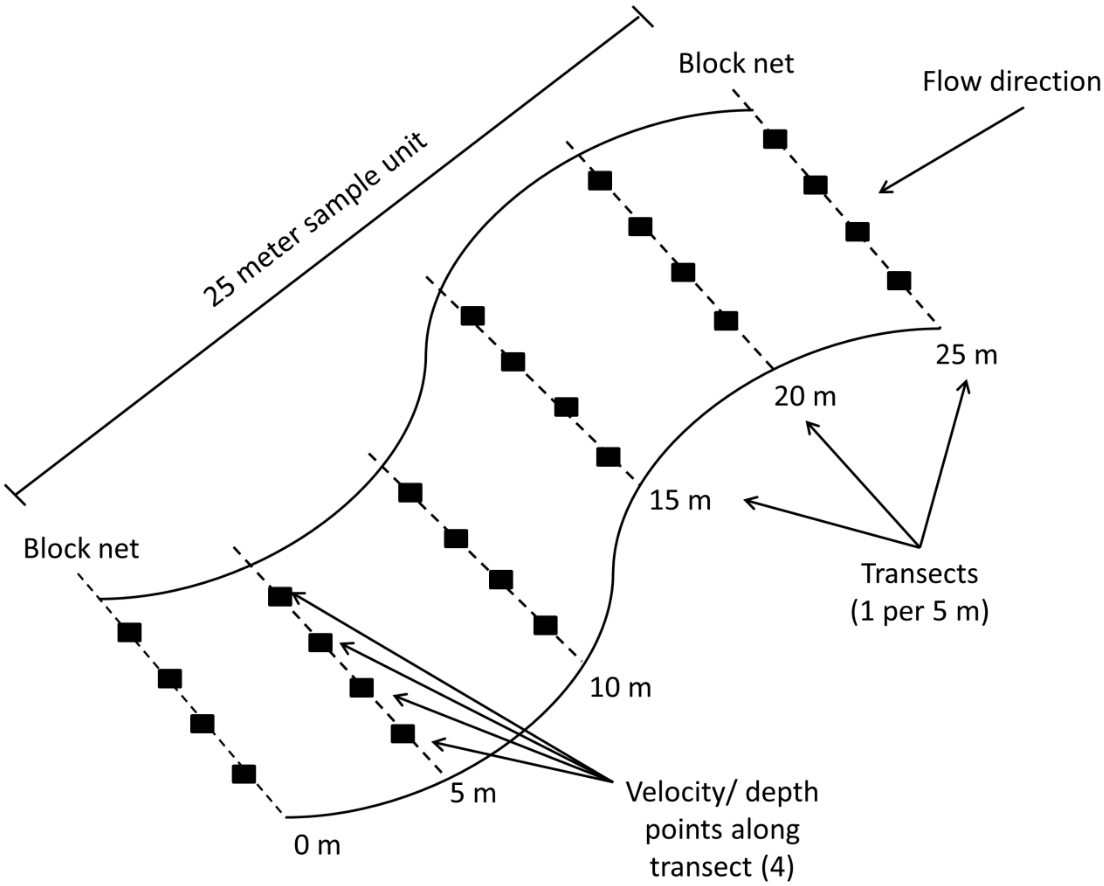

Figure 3. Representation of a 25 m unit with transects spaced every 5 m and points along transects for recording mean wetted width, mean water velocity, and mean water depth data.

At each perpendicular transect spaced every 5 m, mean wetted width, mean water velocity, and mean water depth will be quantified by averaging measurements taken from four locations spaced across the transect at a 1/4th interval level measurement (Peterson and Rabeni 2001). Water depths will be measured to the nearest centimeter using a two meter top-set rod. Water velocities will be measured at 0.6 depth using either a FlowTracker, Marsh-McBirney, or Global Water Flow Probes water current meter attached to a standard, top-set wading rod. The length and wetted widths and depth of undercut bank (if applicable) will be measured using standard measuring tape. The following measurements will be calculated in Program R. The mean cross-sectional area for each 25 m unit will be estimated by averaging the mean wetted width and multiplying it by the mean depth at each transect. The mean volume of each 25 m unit will be estimated by multiplying the channel unit mean cross-sectional area by the channel unit length. The area of each channel unit will be estimated by multiplying a channel unit mean width by length.

Substrate composition will be visually estimated in a 1-meter-wide band centered across each transect and will be categorized as percentages of fine (< 5 mm), gravel (5–50 mm), cobble (50–300 mm), boulder (≥ 300 mm), and bedrock (no particles) (Dunne and Leopold 1978; Peterson et al. 2005). The mean substrate percentages will be estimated for each stream reach by averaging values across transects will be completed in Program R. Between each transect and within the wetted channel, crews will count pieces of woody debris measuring ≥ 1 m long and 10 cm in diameter or as an aggregation of smaller pieces (≥ 1 m long and 10 cm in diameter). The wood density will be estimated by dividing the total number of total wood pieces in a stream reach by the total sample area (length of unit times mean wetted width; Peterson et al. 2005).

Processing of collected materials

At the conclusion of sampling each week, all digital data collection or storage devices will be checked to ensure all data have been removed and archived and their memories cleared. If datasheets were used instead (Appendix C), then all data should be checked for accuracy, reconcile errors, and then entered into Survey123 (SOP 2), and archived in each species-specific and habitat data files for analysis (SOP 3). Complete the metadata (see Element – 5 Metadata; SOP 4). The survey lead should scan the datasheets and then archive the datasheets in the Service Catalog (ServCat; see Element 5 – Data security and archiving; SOP 4).

End-of-season procedures

All data should be proofed for legibility and accuracy and then entered into Survey 123 by the end of each week during the field season. Initial and date each datasheet after entering it into the database and again after proofing the electronic record. Once the data are verified and correct in the electronic database, the data may be read into the Program R to generate annual summaries of the data, population estimates and growth rates, trends, and report species-habitat relationships. This will assist with the production of a document summarizing the field season with the survey dates, counts of the species detected at each stream reach, tables summarizing the environmental conditions, predicted population estimates, predicted population growth rates, and other noteworthy events. The document should be prepared and stored with the season's field datasheets. All field equipment should be cleaned and batteries removed for storage. Additional details on database management and data analysis are included in SOP 3 and 4.

Element 5: Data Management and Analysis

Data entry, verification, and editing

Details for data entry, verification, and archiving can be found in the standard operating procedures (SOPs 2 and 4). Some basic knowledge of Survey123, Microsoft Excel, and Program R are required. Data entry, editing, and reporting of data is to be updated and uploaded into Survey123 and ServCat immediately after survey completion. Data and reports are to be checked by Zone Biologist, Refuge Manager, and Regional Statistician before uploading into ServCat.

Metadata

SOP 4 provides directions on uploading a credit and use limitations file into ServCat. Since Survey123 is used, metadata will be written in ArcGIS Online in the group summary and description. When uploading information to ServCat, the metadata will include updated .csv files that are from the online Survey123 application. This data includes .csv files used in data analysis and photocopied images of datasheets (Appendix C), and any reports produced. All these files will be zipped and uploaded to ServCat. In the ServCat upload, a good description will help future users of the data find the correct files.

Data security and archiving

Historically, the stream survey data was stored on the San Bernardino and Leslie Canyon NWR server. Per the protocol for future surveys, the stream survey data will be stored both on the San Bernardino and Leslie Canyon NWR Server and duplicated in Survey123 for the Rio Yaqui Fish and ServCat. The Survey123 stream reach requires a login, and contains two survey forms for this protocol. The two survey forms are Río Yaqui Fish Depletion Sampling and Río Yaqui Streams Habitat Data. To access these forms one must be a member of the Rio Yaqui ArcGIS online (AGOL) group. The Lead Biologist at San Bernardino and Leslie Canyon NWR will ask the regional data management team to assign specific personnel access to the AGOL group. After each survey year, San Bernardino and Leslie Canyon NWR will upload the final data to the Rio Yaqui Survey123 website, and inform the Regional Data Management Team, Zone Biologist and Statistician that the data upload has occurred.

SOP 4 provides directions for uploading the data to ServCat annually. The regional data management team or NWR staff can upload comprehensive reports at 5 year intervals. The ServCat Reference ID is 104820 for this survey. ServCat is the USFWS's document and geospatial repository. Permission levels in ServCat will be set to Restricted. ServCat can be accessed at: https://ecos.fws.gov/ServCat.

Analysis methods

We use a hierarchical Bayesian approach to fitting formulations of depletion models, providing a natural way to incorporate different structures into the model, incorporating latent variables for modeling, data augmentation, and inferences related to the shape and scale representing the uncertainty in the posterior probability distribution of the model parameters (Gelman 2006). In general, hierarchical Bayesian models are adaptable to various capture recapture experiments, such as depletion models. In this analysis we consider the design of the depletion survey by assuming that animals are captured from I spatially distinct subpopulations within the K year on J different sampling occasions, and populations are demographically closed to changes in abundance, births, deaths, immigration, or emigration at the time of sampling.

We consider the experimental design where the observed elements of the model represent the sequence of counts of unmarked individuals, y, from each sampling occasion $j = 1, \ldots J$ within each set of $i = 1, \ldots, I$ subpopulation for year $k = 1, \ldots, K$. Therefore, the observed data, y_{ijk}, can be denoted by the matrix of observed numbers of animals during the survey as $Y = \{y_{ijk}: i = 1, 2, \ldots, I; j = 1, \ldots, J; k = 1, \ldots, K\}$ and is regarded as a binomial outcome $h(y_{ijk}|N_{ijk}, q_{ijk})$ (or multinomial; Dorazio et al. 2005), as

$$L(q_{ijk}, \lambda_{ijk}; \{y_{ijk}\}) = \prod_{i=1}^{I}\prod_{k=1}^{K}\left\{\sum_{N_{ik}=max_{y_{ijk}}}^{\infty}\left(\prod_{j=1}^{J} Bin(y_{ijk}; N_{ik}, q_{ijk})\right) f(N_{ik}; \lambda_{ik})\right\}$$

The outcome is conditional on the unknown total number of individuals available for sampling, N_{ijk}, within subpopulation i of year k, where the infinite summation is replaced over N_{ik} by a summation of observation. Moreover, depletion surveys require the removal of captured individuals during occasion j. Under this specification, q_{ijk}, is defined as the probability of detecting animals during the j^{th} removal from the i^{th} subpopulation and k^{th} year, given that they have not been collected in earlier removals (Zippin 1956; Royle and Dorazio 2006). That is

$$y_{ijk}|y_{i1k}, y_{i2k}, \ldots, y_{i,j-1k}, N_{ijk}, q_{ijk} \sim Bin(N_{ijk}, q_{ijk})$$

where $N_{i1k} = N_{ik}$ and

$$N_{ijk} = N_{ik} - \sum_{j=1}^{j-1} y_{ijk}$$

for $j = 3, 5, \ldots, l$. A standard approach is to assume a Poisson distribution for the latent abundance state, $[N_{ik}|\lambda_{ik}] \sim Poisson(\lambda_{ik})$, where λ_{ik} is the expected abundance of animals within subpopulation i, for $i \in I$ and year k, for $k \in K$. Since the abundance, N_{ik}, at a stream reach varies, we specified our model to account for overdispersion. Previous approaches have used simple distribution assumptions of the prior distribution to account for stochastic sources of variation in the abundance parameter among stream reaches by specifying the process model for N_{ik} to be marginal to a hierarchical element, ε_i. The dispersion parameter of the hierarchical element is integrated into the likelihood of the Poisson process as a random effect to account for the variation among stream reaches, resulting in a marginally distributed multi-subpopulation negative binomial mixture by considering $f(N_{ik}|y_{ijk}, \varepsilon_i)$, $\varepsilon_i \sim gamma(\theta, \theta)$, which results in a probability distribution (P) for N_{ik} as:

$$P(N_{ik} = n_{ik}|\lambda_{ik}, \theta) = \frac{\Gamma(n_{ik} + \theta)}{\Gamma(n_{ik} + 1)\Gamma(\theta)}\left(\frac{\lambda_{ik}}{\lambda_{ik} + \theta}\right)^{n_{ik}}\left(\frac{\theta}{\lambda_{ik} + \theta}\right)^{\theta}$$

The parameter θ is positive and large values of θ being consistent to variability similar to the Poisson distribution. Thus, as $\theta \rightarrow \infty$, the distribution of N_i converges to a Poisson random variable, where the level of dispersion (θ) is assumed to be the same among all I subpopulations and K years, providing a natural hierarchical extension of the binomial-Poisson mixture (Stewart and Long 2016; Stewart et al. 2017).

We modeled the detection probability process by assuming that it decreased after the first pass due to changes in sampling effort, animal behavior, emigration or immigration (Cross and Stott 1975; Peterson and Cederholm 1984; Riley and Fausch 1992). Therefore, we specified the detection probability to vary by stream reach and successive depletion passes as $q_{ijk} = p_{ik}(1-p_{ik})^{j-1}, p_{ik} \sim beta(a,b)$, formally specified as $p_{ik} = p_1 + (p_2 - p_1)(1 - a^{j-1})$, which assumes that catchability declines monotonically across successive passes (Schnute 1983; Dorazio et al. 2005).

We specified the parameter model of the binomial-Poisson mixture models to relate habitat covariates to the constant detection probability through a logistic link function from the best-forming variable detection function from the simulation analyses described in 2.2. For example, assuming that the detection probability is constant across successive passes, we specified the mixture model to relate habitat covariates to the detection probability, as:

$$logit(q_{ijk}) = \gamma_0 + \sum_{v=1}^{w} \gamma_v x_{v,ik}$$

However, because detection probability likely varies across successive passes, then we specified the detection model to vary using one of the best performing of the two detection models described in 2.2. For example, specifying the detection function as $q_{ijk} = p_{1ik} + (p_{0ik} - p_{1ik})(1 - a_{ik}^{j-1})$, then we used a logit-link function to relate habitat covariates to the detection probability, as:

$$logit(p_{ik}) = \gamma_0 + \sum_{v=1}^{w} \gamma_v x_{v,ik}$$

Mean abundance was specified using a log link function, as:

$$log(\lambda_{ik}) = \alpha_0 + \sum_{v=1}^{w} \alpha_v x_{v,ik} + \delta_k + \epsilon_h$$

where x_v are predictors $v = 1,2,..w$ such as water depth, net depth, water temperature, and percent submergent aquatic vegetation measured at a subpopulation i within year k. The $\gamma's$ and $\alpha's$ are the intercept and slope parameter estimates. We added a random effect or exchangeable error term δ_k and ϵ_h that specifies the variation in mean abundance among the k^{th} year and h^{th} watershed units.

Software

Multiple software programs facilitate the collection, processing, storage, and analyses of the data collected during this monitoring effort. Recommended software and their sources are:

- Microsoft® Excel 2010, www.microsoft.com
- Program R, https://www.r-project.org/
- R Studio, https://www.rstudio.com/
- JAGS, http://mcmc-jags.sourceforge.net/
- R package, `jagsUI`, https://cran.r-project.org/web/packages/jagsUI/index.html
- R package, `dplyr`, https://cran.r-project.org/web/packages/dplyr/
- R package, `ggplot2`, https://cran.r-project.org/web/packages/ggplot2/index.html
- R package, `devtools`, https://cran.r-project.org/web/packages/devtools/index.html
- R package, Depletion, https://github.com/drstewart11/Depletion

Note: *`jagsUI`, `dplyr`, and `ggplot2` will be downloaded as dependencies with R package "`Depletion`". Therefore, open R Studio (after installing Program R) and either install*

(`install("devtools")`) or load R package `devtools` to your machine if it was previously installed (`library(devtools)`). Next, install (`install_github("dstrewart11/Depletion")`) or load (`library(Depletion)`) to your machine. Depending on whether you install or load, R packages `jagsUI`, `dplyr`, and `ggplot2` will be automatically downloaded or required.

Element 6: Reporting

Implications and application

The primary objective of this monitoring effort is to provide reliable estimates of abundance for assessing species status and progress towards downlisting criteria (USFWS 2007), while also developing spatially-explicit resource use models that can facilitate land conservation as well as species range expansion through the delineation of important habitats at stream reaches not known to previously house Río Yaqui fishes.

Additionally, regular and timely dissemination of survey results is essential for making informed management decisions. Summarizing stream fish survey data will help determine if management objectives are being met and will be used to assess the capacity of the monitoring efforts to detect trends prescribed in objective 1. Interim reports should be prepared annually for the purpose of summarizing and interpreting depletion data for each species and stream reach; whereas comprehensive reports should be submitted every five years. The depletion survey data should be submitted to the Project Leader at the land unit and also to the Regional Statistician and Zone Biologist for review and assessment. The USFWS encourages publication of significant findings in scientific journals or USFWS publications (USFWS 2007).

Objectives and methods for reporting

The annual interim reports will consist of a brief summary of survey activities and results designed to update stakeholders and USFWS personnel. These reports are not intended to be comprehensive. Instead, these reports are intended to provide year specific information of sampling activities. Summaries should include stream reach-specific estimates of the number of depletion passes, number of seconds per pass, backpack electroshocking settings used at each stream reach, and habitat features. The report should also include species-specific summaries at each stream reach, such as the number of individuals of each species captured, and estimates of detection probability and true abundance with associated 95% credibility intervals. One interim report will be issued after the September–October survey period.

Comprehensive reports will be comprised of a complete account of monitoring efforts for Río Yaqui fish in streams. These reports will be submitted every five years, and will describe background information and survey objectives, briefly describe survey methodology, provide details of data analyses, report results, provide comparison with previous years and report trends, discuss important findings, and provide context for management and planning decisions. Any and all deviations from protocol should be avoided.

Summary of results

Reports will include stream reach- and species-specific summary statistics and their associated variation. Stream reach-specific summary statistics are:

1. The number of depletion passes.
2. Number of seconds per pass (report mean, range, and standard deviation).
3. Backpack electroshocking settings used at each stream reach.
4. Habitat features (see Environmental Covariates).

In addition to these stream reach-specific features, species-specific estimates for each stream reach will contain:

5. The number of individuals of each species captured.
6. Detection probability estimates (including 95% CI).
7. True abundance estimates (including 95% CI).

Those summary statistics should be reported in both the interim and comprehensive reports. However, comprehensive reports will also contain:

8. Intercept and slope parameter estimates and associated 95% CI for the modeled habitat variables (both alpha's and beta's) for each species.
9. Stream reach-specific population growth rate for each species to assess trends.

The intercept and slope parameter estimates describing the relationship between Río Yaqui fish abundance and environmental covariates will further our understanding of how habitat controls population growth of these species. These covariates are included in a priori models and should be described in the comprehensive reports and summarized as described above (#8).

Important findings

The comprehensive reports should include a section aimed at discussing the implications of the survey results, and how they relate to the survey objectives and relevant management decisions. For example, the survey results may identify important habitat features controlling population growth rate for each species that can then be used to trigger a management response. Additionally, the survey results may identify a set of habitat features that are similar in comparison to habitat measurements at previously uninhabited stream reaches, such that future translocations occur to increase the number of subpopulations for each species. The discussion should also address the survey results, conclusions, and also any recommendations for changes in management strategies. If a management response is identified or an alternative recommended, include additional information that documents how these results will be useful to management.

Reporting schedule

One interim report will be issued each year and comprehensive reports will be issued every five years.

Report distribution

Results should be discussed with the Refuge Manager and Regional Statistician. The regional data management team will upload the data into ServCat once final, comprehensive report is complete. Copies of the interim and comprehensive reports should be archived at the refuge station and ServCat, and distributed to all interested partners.

Element 7: Personnel Requirements and Training

Roles and responsibilities

A minimum of two individuals are needed to conduct the survey. Crews will consist of a Lead Biologist (Refuge Biologist). This individual is responsible for implementing the monitoring program, training additional crewmembers, implementing the survey protocol, data entry, data proofing, and quality control. The Lead Biologist must be Department of Interior-Electroshocking certified. Additional crewmembers will be responsible for assisting the Lead Biologist with coordinating logistics. Data analysis will be conducted by the Regional Statistician or trained and qualified biologist. Compilation of interim and comprehensive reports will be a collaborative effort among the Lead Biologist, Regional Statistician, Regional Data Manager, and Refuge Manager (see Element 6 – Reporting).

Qualifications

The Lead Biologist will be responsible for training additional crewmembers. Crewmembers must feel comfortable with sampling and wading in streams, equipped with personal protective equipment, understand safety protocols, and be familiar with electroshocking equipment. Crewmembers must have the ability to hike in rough terrain, lift at-least 50 lbs, and have the ability to endure 5 hours or more of working outside. All staff involved in conducting, coordinating, and analyzing data from these surveys must conduct monitoring activities with scholarly and scientific integrity (USFWS 2011).

Training

The Lead Biologist must have the required electroshocking training outlined in USFWS Service Manual, 241 FW 6 (https://www.fws.gov/policy/241fw6.html#section_6_4). The training courses needed are Principles and Techniques of Electroshocking (Online) and Electroshocking Safety. More information regarding these and other electroshocking training courses are available at the NCTC website (https://training.fws.gov/). Additionally, at-least two crewmembers need to be CPR/First Aid certified in case of emergencies.

It is important that all crewmembers are familiar with the electroshocking equipment and their personal protective equipment.

Scientific collecting permits and HACCP

The Refuge Manager and Lead Biologist must have the required federal and state scientific collection permits. The federal collection permit authorizes activities with federally listed species on federal lands. The state collection permit authorizes activities of all “identified” non-federally listed species (Appendix D). Additionally, the Lead Biologist must adhere to all reporting requirements associated with each permit. Moreover, this activity is also associated with a management tool to identify risks and focused procedures to minimize the risk of moving potentially invasive species during survey activities. These steps should be reviewed prior to the beginning of each field season and reviewed prior to each sampling occasion. The Hazard Analysis Critical Points (HACCP) planning document is included in this protocol (Appendix E).

Element 8: Operational Requirements

Budget

The costs to complete the implementation of this protocol are divided into several categories (i.e., Fuel to the Refuge, Staff Costs, Survey Equipment, and Office Supplies; Table 4). Costs associated with Staff Costs considered with and without a permanent technician to assist the Lead Biologist. Staff Cost also included time spent formatting and analyzing the data by the Regional Statistician. The largest line item cost of the survey was Staff Costs. Equipment cost related to the initial supplies and any reoccurring supplies needed to maintain equipment and attain additional batteries.

Table 4. Estimated budget for monitoring Río Yaqui fishes in streams in and around San Bernardino and West Turkey Creek, AZ.

Budget item	Estimated Cost w/only Biologist	Estimated Cost w/Technician and Biologist
Fuel to the Refuge	$300	$300
Staff Costs[1]	$15,720	$15,720
Survey Equipment		
Initial Supplies	$11,000	$11,000
Reoccurring Supplies (e.g., batteries, replaced equipment, Miscellaneous)	$1,500	$1,500
Office Supplies	$1,000	$1,000
Survey Costs		
Initial Survey	$25,515	$29,520
Follow-up Surveys	$14,515	$18,520

Staff time

The total staff time required to complete training, survey preparation, data collection, data processing, data analysis, and reporting and distribution have been estimated from the approximate time required to complete this survey in 2016-2018. The time to complete this protocol will likely decrease as the methods, data management aspects, and report writing becomes more familiar. Staff time is contributed to a single Lead Biologist and volunteer and also a Lead Biologist and Technician, as well as the Regional Statistician. The estimated Full Time Employee (FTE) equivalence to complete this survey is 0.14.

Schedule

Monitoring efforts will occur annually between September–October. To ensure that the survey is performing correctly it is expected that data processing, data analysis, and reporting will be conducted within three months after the completion of the September–October surveys for interm reports and 6 months compressive reports. Interim reports will be issued once annually and comprehensive reports will be issued every five years. The interim reports are of lowest priority and considered optional given time constrains of USFWS personnel. Comprehensive reports are not optional and must be completed in a timely manner (i.e., every five years).

Coordination

The Lead Biologist (i.e., Refuge Biologist) will be coordinating all monitoring activities. The Lead Biologist will need to coordinate with other offices and stakeholders to attain additional crewmembers, data analyses through the Regional Statistician or Zone Biologist, and data management with the Regional Data Manger. The Lead Biologist will typically be the Refuge Biologist. The Assistant Refuge Manager or the Refuge Manager may be the Lead Biologist in the absence of a Refuge Biologist.

Element 9: References

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Standard Operating Procedures

SOP 1: Survey Logistics

UNDERSTANING THIS DOCUMENT

- Emboldened terms are commands, tools, or tasks within the referenced software programs (i.e., Microsoft Excel 2010, Program R).
- Italicized text indicates background information, a filename, or a field name.
- SOP written for a Windows 10 environment.

DATA COLLECTION

This SOP will be reviewed by the observers prior to each survey period.

- PRE-SURVEY LOGISTICS FOR DATA COLLECTION

  Before leaving Refuge Headquarters

  1. Read and familiarize yourself with Element 3 and 4.
  2. Plan surveys in advance.

     Note: *Backpack electroshocking should not be attempted if the average depth of water is too deep for operators to wade at less than "thigh depth" for the majority of the exercise (e.g., Black Draw at Leslie Canyon NWR). Suspend wading operations if adverse weather or water conditions are a safety concern (i.e., thunder, lightning, swift water). Lastly, do not enter the water if you are unable to swim or are uncomfortable with your swimming abilities.*

  3. **Review** and **Print** Table 3 in Element 4. Table 3 should also be used as a checklist to ensure that all items are accounted for and loaded in field vehicles prior to leaving the office.
  4. It is important that staff check (and then double check) all personal protective equipment and survey equipment at-least two weeks in advance of the planned survey. <u>For example, staff could **identify** and **repair** leaks in any and all waders, repair nets, and also the nylon mesh basket of each dip net prior to conducting surveys each year.</u>
  5. **Ensure** water quality meters are **calibrated** following procedures identified in their manual, test equipment (including the backpack electroshocking device), and begin assembling the equipment so that it can be readily found prior to conducting surveys each year. In doing so, surveys become more time efficient, one is proactive in preventing bodily injury, and increases the integrity of the data being collected.
  6. **Ensure** all electronic equipment is fully charged or has new batteries installed. Charge electroshocking batteries the night before the survey.
  7. It is important that staff is familiar with all the equipment and how to operate. Staff should also discuss all aspects of the survey protocol.

After leaving headquarters – establishing a sample reach

1. As mentioned previously, stream reaches known to house Río Yaqui fish species (and nonnative species) and those located downstream are already established within the San Bernardino NWR and El Coronado Ranch (West Turkey Creek, AZ) sampling units. Their coordinates are: Leslie Creek (n = 2; Site 1 = UTM(12) 0641482, 3495950; Site 2 = UTM(12) 064163, 3496076); Minckley (n = 1; Site 3 = UTM(12) 0665030, 3468928); Twin Site (n = 1; Site 4 = UTM(12) 0665172, 3468611); West Turkey Creek, AZ Site 5 = UTM(12) 0651053, 3526946; Site 6 = UTM(12) 0651034, 3526946; Site 7 = UTM(12) 0650992, 3526429; Site 8 = UTM(12) 0650877, 3526951; Site 9 = UTM(12) 652787, 3526648; Site 10 = UTM(12) 652810, 3526663).
2. At each stream reach, crews will **establish** a 25 meter sampling unit from the center of the sampling location by working along the bank edge and staying out of the water. First, the crew should select and mark off the sample reach start and end points with flagging tape. Use the measuring tape to ensure that each reach is 25 m.
3. Second, block nets (mesh size ≤ 3 mm) should be stretched at the start and end of the 25 m reach (i.e., across the upstream and downstream section of the stream).

   Note: Do not set up block nets at upstream or downstream locations having an undercut bank. If encountered, **increase** the length of the stream reach by 5 meters (25 m to 30 m), and then **check** for undercut bank. If no undercut bank, then **set** the block net across the stream. If the undercut bank is present, then **increase** the length of the stream reach to be sampled by 5 m (30 m to 35 m), check for undercut bank, and then either set the block net or continue increasing the length of the stream reach. **Record** the final length of the stream reach sampled on the data sheet.

4. Each net should be **visually inspected** to ensure that the side of the net extends beyond the wetted width of the stream channel, the top of the net resides 12 inches above the water surface, and it is recommended that you weight the bottom net using a rock to ensure that the net stretches from the water surface to the substrate.

   Note*: This is one of the more critical steps because immigration and emigration of fish will lead to biased abundance, detection probability, and species-habitat relationships.*

5. **Use** the GPS (e.g., Garmin) to determine the latitude and longitude in decimal degrees at the upstream block net.
6. **Record** the GPS reading, along with stream name, station ID (from previously prepared sampling unit list), date, and crewmember names on the data sheet.

Water quality measurements

7. **Measure** water quality characteristics using **<u>calibrated</u>** YSI meters at each study reach for each sampling occasion downstream of the downstream block net.

   a. To measure water quality, **lower** the probe into the stream, and then allow the probe to equilibrate. When field measures no longer appear to be changing, **record** water temperature to the nearest 0.1 degree Celsius (°C), pH to a value of 0.1, specific conductance to 0.01 microsiemens per centimeter (µs/cm), dissolved oxygen (DO) to the nearest 0.01 milligram per liter (mg/L), turbidity (NTU), and total algal records on the printed data sheet or in the tablet computer

Calibrating the electroshocking unit

8. Make sure prior to assembly that the electroshocking unit is turned to the off position.

   Note*: The unit is off when the red knob on the top is rotated completely counter clockwise.*

   a. **Assemble** the electroshocking unit by first securely placing a freshly charged battery within the backpack unit. Properly attach the anode (pole with aluminum ring) and the cathode (black rat tail) to their respective output connectors on the bottom rear of the instrument case of the electroshocking unit, and then replace the battery compartment cover and re-secure the latches. A crewmember should then help place the backpack on the individual who will be electroshocking. Turn on the unit.

9. Using the water quality values, **determine** the initial voltage setting selected.

   a. In low conductivity water (<100 µs), high voltage (900-1000) and low amperage are needed, while in high conductivity water (>300 µs), low voltage (100-400) and high amperage are needed. Values of conductivity 100-300 µs require moderate voltage (700-800) and amperage.
   b. To change these values in the Smith-Root electroshocking (currently being used) unit, **press** the up arrow until the waveform is displayed to view the current values.

10. If these settings are not desired based on the conductivity values, **press** the Pulse Type button and use the up/down arrow keys to **select** Standard Pulse; **press** Enter.
11. Next, **press** the volts key and use the up/down arrow keys to **enter** the desired voltage following the recommendations above; **press** Enter.
12. **Press** the Freq key and use the up/down arrow buttons to set the desired frequency, which should range between 40-60 Hz for small-bodied cyprinids. **Set** the initial frequency to 40 Hz; **press** Enter.

13. **Press** the Duty Cycle button and use the up/down arrow buttons to set the duty cycle to 12%; **press** Enter.
14. **Press** the Power Limit button and use the up/down buttons to set the power limit to 400 watts; **press** Enter.
15. **Place** the anode ring and cathode cable in the stream located upstream from the upstream block net. **Press** the anode pole switch and listen to the audio alarm.

    ***Note**: If the alarm is beeping on and off 1 time per second, release the anode pole switch and increase the output by 50 volts.*

16. If the tone is beeping on and off two or more times per second, **release** the anode pole switch and commence electroshocking (see below). If not, **repeat** this step by increasing the voltage by 50 volt increments until the audio alarm beeps two or more times per second.

    ***Note**: Test fish response to electroshocking parameters. Settings high enough to quickly subdue fish should be avoided, as these settings result in higher injury rates. In general, if it takes more than 5 seconds for fish to recover, the frequency, duty cycle, or voltage of the electroshocking unit needs to be adjusted. To do so, press the Volts button and use the up arrow button to increase or decrease voltage by 50 volt increments; press Enter.*

17. The following are optional instructions in the event that one may advance through these steps several times and reach the maximum (400, 800, or 1000V which depends on your conductance) voltage with fish responding weakly to the electrical field. If this happens, return the voltage to the original setting as determined by the conductivity parameters above, then proceed to increase the duty cycle by 10% from the original value; **press** Enter and try again.
18. **Repeat** steps 16-23.
19. If the maximum of voltage is reached again, then **return** the voltage to the original setting as defined above and continue to increase the duty cycle by additional 10% increments.
20. **Repeat** above steps 16-23 by increasing voltage until fish respond to the electrical field.
21. Continue repeating these steps until the fish respond to the electroshocking unit or until the duty cycle is set to 50%.

    ***Note**: If fish still do not respond after following the above steps and at 50% duty cycle and 400V, reduce the duty cycle back to 12%, return volts to the initial setting, and increase the frequency by 10 Hz (press the Freq button, then the up arrow); press the Enter button and test the fish response. If fish continue to not respond, repeat the above steps using the higher frequency values (increase voltage and duty cycle values). Test fish response. Then increase the frequency value by 10 Hz and repeat. Frequency should not be set higher than 60 Hz.*

22. **Record** the settings used to sample each stream reach in the datasheets or tablet computer.

- SURVEY LOGISTICS FOR DATA COLLECTION

Depletion sampling

1. **Set** the 10 buckets equipped with their own aerator on the shoreline adjacent to the sampled stream reach.
2. Use flagging tape or write on the buck to identify each bucket as 1 to 10.
3. **Zero** the time on the left side of the electroshocking unit.
4. **Zero** the stopwatch being used by the netter.

   Note*: The number of seconds per pass should be approximately 300 seconds. The netter is responsible for keeping track of time and instructing the lead biologist of time during sampling to maintain a constant amount of effort among passes.*

5. Crewmembers should enter the stream reach at the downstream block net, but only after the electroshocking settings have been set following the SOP.
6. **Initiate** electroshocking and proceed upstream with bank-to-bank sweeping of the anode pole and dip net (mesh size ≤ 3 mm).

   Note*: This sweeping technique is used to ensure maximum coverage of the area being sampled, including insuring that all available habitats are properly targeted.*

7. Captured fish should be **placed** into one of the uniquely identifiable buckets that coincide with the current pass.
8. Once the crew reaches the upstream block net, the anode should be run along the entire length of the block net using a wafting technique to draw stunned fish away from the net and pulled towards the crew.
9. The netter should **sweep** the upstream block net several times to ensure that all captured fish that retreated to the net have been removed.
10. **Stop** the timer on the personal watch (e.g., stop watch).
11. **Place** the uniquely identifiable bucket (designated for the specific depletion pass: 1-10) containing stunned fish on the shoreline, the aerator turned on, and fish visually inspected to ensure quick recovery.

    Note: *If fish are not doing well in the bucket when released from the net then surveys should be halted and the settings on the electroshocking unit decreased.*

12. **Record** the number of seconds per pass as identified on the electroshocking unit either on the data sheet.
13. **Zero** the time on both the electroshocking unit and stop watch.

14. **Inspect** both block nets to ensure the nets remain stretched from bank-to-bank and stretched from 12 inches above the water surface to substrate.
15. After 5 minutes, the netter should then grab the bucket required for the next pass.
16. **Wade** into the stream reach by entering the stream at the downstream block net, start the timer on the personal watch to keep track of pace, and begin collecting stunned fish using the bank-to-bank sweeping of the anode pole and moving in an upstream direction.
17. Steps 3 to 16 should be **repeated** a minimum of 5 times, and then these steps will be **repeated** until a zero or one is observed on at-least two successive passes after the initial 5.

    a. Sampling will conclude if a zero or one count is observed on the 4th and also the 5th pass. Or if a 0 or 1 count is observed on passes 6 and 7. However, if a species count is >1 is observed on either pass 6 or 7, then the crew will continue sampling for an additional pass. If during this additional pass (say passes 7 and 8 or passes 8 and 9) a zero or one is not observed on two consecutive passes, then continue sampling until a total of 10 passes have been completed following Steps 3 to 16.

    ***Note**: Please remember to keep notice of the number of seconds per pass and visually inspect each block net before and after each depletion pass.*

18. **Enumerate** and **record** the number of fish captured for each pass and species, and return each individual to their bucket identified for the pass that they were captured.

    ***Note**: It is critical to not sum the total number of individuals captured. One must record the total number of each species captured for pass 1, pass 2, and so on.*

19. Once the catch data is recorded on the data sheet, leave the fish in their respected bucket until after habitat is measured, and then **redistribute** all of the captured fish to the stream channel from which they were captured.

Habitat data or environmental covariates

20. Immediately after depletion sampling, and using a line-transect method, **establish** transects perpendicular to flow every 5 m starting from the downstream block net toward the upstream block net of the 25 m stream reach (see Figure 3).
21. At each perpendicular transect spaced every 5 m starting with measurements from the downstream block net, water velocity and water depth will be quantified from measurements taken from four locations across the transect (Peterson and Rabeni 2001).
    a. **Measure** water depths to the nearest centimeter using a two meter top-set rod. **Measure** water velocities at 0.6 depth using either a

FlowTracker water current meter attached to a standard, top-set wading rod.

b. **Measure** the length of the stream reach (25 m) and wetted widths using standard measuring tape (nearest cm).
    i. Wetted width is the representative width of the wetted stream at the location of each transect (see Figure 3 and 4).

Figure 4. Representative image showing wetted width of a stream channel that is below bankfull. Image is from the Department of Environmental Resources Engineering (www.fgmorph.com/fg_3_10.php).

c. **Estimate** the mean cross-sectional area for each 25 m unit by averaging the mean wetted width and multiplying it by the mean depth at each transect. This will be completed in Program R at the time of analysis.

d. **Estimate** the mean volume of each 25 m unit by multiplying the channel unit mean cross-sectional area by the channel unit length. This will be completed in Program R at the time of analysis.

e. **Estimate** the area of each channel unit by multiplying a channel unit mean width by length. This will be completed in Program R at the time of analysis.

22. **Estimate** the substrate composition in a 1-meter-wide band centered across each transect.
    a. This requires two individuals standing shoulder to shoulder, centered across each transect (Figure 3), facing perpendicular to the 5 m transect, and then having both visually estimate the percent substrate composition per category (see below c) across the 1-meter-wide (i.e., shoulder to shoulder wide band).

b. In the event that the visual estimates per category do not agree between the two individuals, then both should resolve any differences before agreeing on a final estimate.

c. **Categorize** substrate as percentages of fine (< 5 mm), gravel (5-50 mm), cobble (50-300 mm), boulder (≥ 300 mm), and bedrock (no particles) (Figure 5; Dunne and Leopold 1978; Peterson et al. 2005). **Estimate** the mean substrate percentages for each unit by averaging values across transects. This will be completed in Program R at the time of analysis.

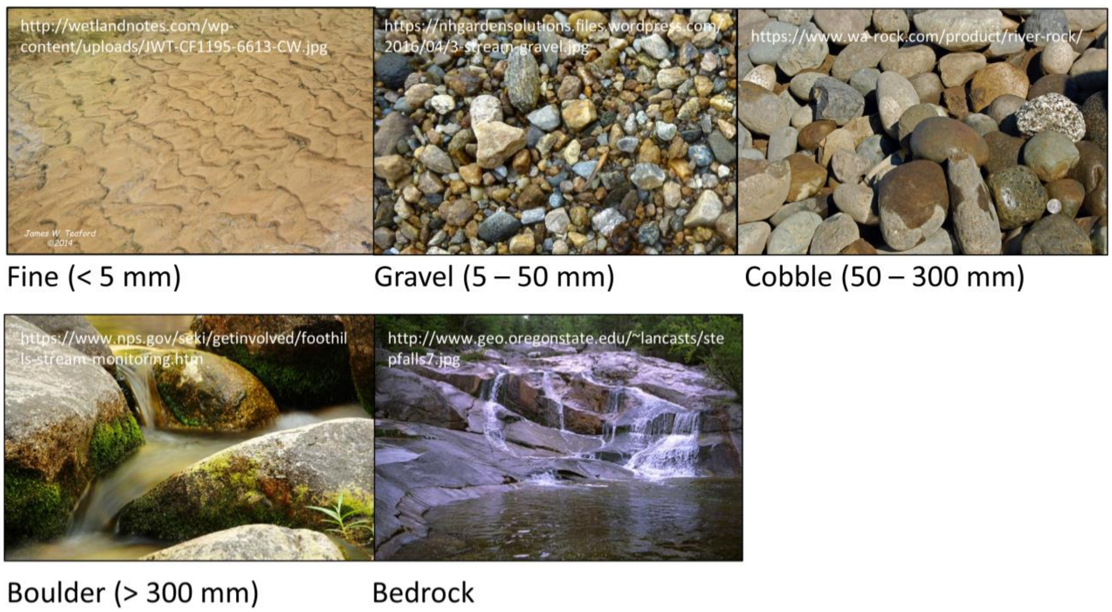


Figure 5. Substrate types and their associated measurements. Pictures were derived from various internet sources.

23. Between each transect and within the wetted channel, crews will **count** pieces of woody debris measuring ≥ 1 m long and 10 cm in diameter or as an aggregation of smaller pieces (≥ 1 m long and 10 cm in diameter).

    a. **Estimate** the wood density by dividing the total number of total wood pieces in a unit by the total sample area (length of unit times mean wetted width; Peterson et al. 2005).

SOP 2: Post-survey Data Processing and Formatting

UNDERSTANING THIS DOCUMENT

- Emboldened terms are commands, tools, or tasks within the referenced software programs (i.e., Microsoft Excel 2010, Program R).
- Italicized text indicates background information, a filename, or a field name.
- SOP written for a Windows 10 environment.

DATA PROCESSING

This SOP will be reviewed by the observers after each survey period.

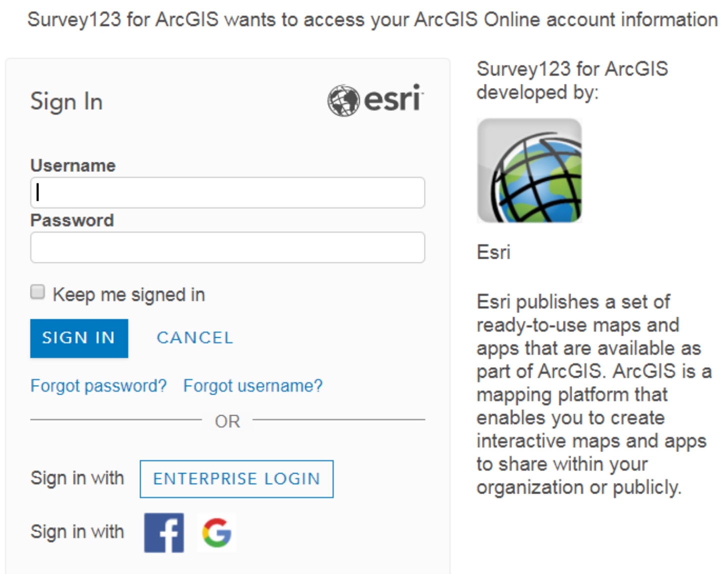
- POST-SURVEY LOGISTICS FOR DATA PROCESSING
    1. **Read** and **familiarize** yourself with Element 4 and 5.
    2. At the conclusion of each field season all digital data collection or storage devices will be **checked** to ensure all data have been removed and archived and their memories cleared.
    3. If paper datasheets were used instead, then all data should be checked for accuracy and archived in each species-specific and habitat datafiles for analysis.

       ***Note**: These data from the datasheets should be entered into Survey123. If you do not have access to the Rio Yaqui group in ArcGIS Online or Survey123, contact the Regional I&M Data Manager. Once the data are entered into Survey123, save the data to your local Río Yaqui fish stream working directory and then uploaded to ServCat.*

    4. There are two Survey123 forms that will be used for data entry. The forms are for the Rio Yaqui Fish Depletion Sampling and the Rio Yaqui Streams Habitat Data. To input the data into the Sample Depletion form, go to the Survey123 website, https://survey123.arcgis.com, and login, or the following link that takes you directly to the Survey 123 page,

       https://survey123.arcgis.com/share/39f41e460a3e4809bf5313ee667dc7d5

5. **Select** ENTERPRISE LOGIN



6. **Enter** fws as your ArcGIS organization's URL. **Select** continue.
7. **Select** U.S. Fish and Wildlife Service.
8. Once you are logged in **click** My Surveys.
9. **Select** the Rio Yaqui Fish Depletion Sampling survey form.
10. **Select** the stream reach sampled from the list. Stream reach names are pre-programmed. If a stream reach name is not identified, then **select** other and **enter** the stream reach name.
11. **Enter** Easting and Northing coordinates, and the UTM Zone of the stream reach.
12. Next, **Enter** the count data from pass 1 to 10 for each species. In Survey123, box Count1 captures the number of each species captured on the first pass, and so on for the remaining boxes Count2 to Count10.
    a. GIPU is the count file for Yaqui Chub.
    b. POSU is the count file for Yaqui Topminnow.
    c. AGCH is the count file for Mexican Longfin dace.
    d. Seconds is the number of seconds that it took to complete the first depletion pass.

    ***Note**: Enter the numerical value representing the number of fish observed (i.e., 1, 2, …., Infinity) or not observed (i.e., 0) at the sampled stream reach. If the stream reach was not sampled in a given year, then input NA for each pass for each species. However, and due to the small number of stream reaches required to sample, all stream reaches should be sampled annually and any discontinuities in sampling effort and the number of sampling stream reaches should be avoided.*

13. **Repeat** Step 11 for boxes Count2 to Count10.

Note: *The fields in each CountX box are required to have either a NA or a number. In the event that only 5 depletion passes were needed to deplete the local populations of each species at a stream reach, as an example. Then, input NA into the species-specific fields and the number of seconds for the remaining 6 to 10 depletion passes. Do not enter a zero.*

14. Next, **Visit and Select** the Rio Yaqui Streams Habitat Data sheet using the following link:

    https://survey123.arcgis.com/share/76a051a92a024eb5a4bd311387aacf54

    or

    by selecting the Rio Yaqui Streams Habitat Data survey form in Survey123, https://survey123.arcgis.com

15. **Select** the stream reach sampled from the list. Stream reach names are pre-programmed. If a stream reach name is not identified, then **select** other and **enter** the stream reach name.
16. **Enter** Easting and Northing coordinates, and the UTM Zone of the stream reach.
17. **Identify** if the Subsystem for the stream is Perennial or Intermittent by checking one or the other box.
18. **Enter** into the Wood Pieces field the number of woody debris pieces counted in the surveyed stream reach.
19. **Enter** into the NumberNets field the total number of dip nets used to capture fish during the survey.
20. Following Figure 3, **Identify** the Channel Unit type (1 = Riffle, 2 = Run, and 3 = Pool) for each the 6 transects spaced every 5 m from the downstream to upstream block net of the 25 m stream reach.

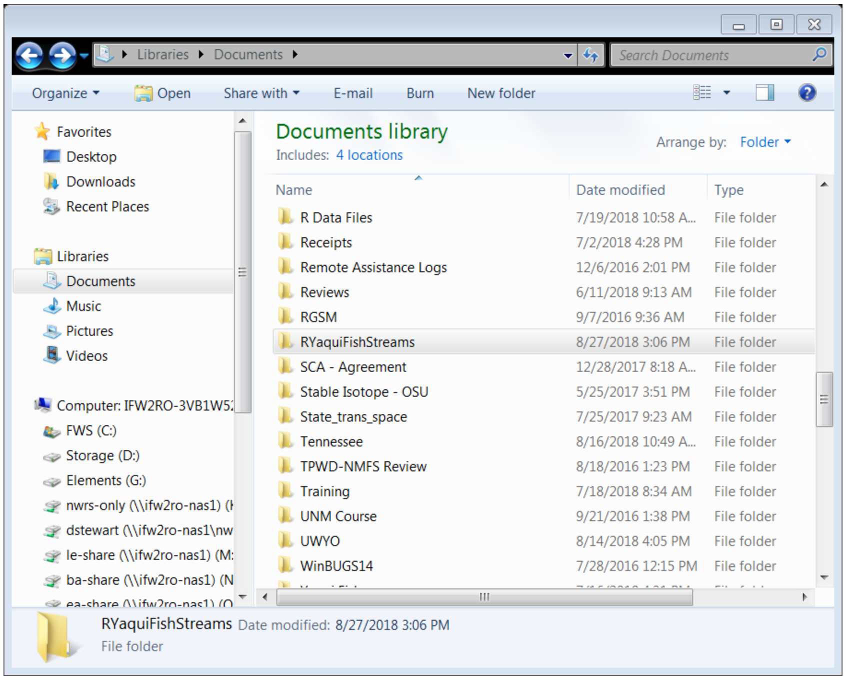
    Note: *CHUnit_0 identifies the downstream block net, CHUnit_5 to CHUnit_20 are the subsequent 5 m transects, and CHUnit_25 is the upstream block net.*

21. Following Step 21, **Enter** the measured channel width (CHWidth) for each of the 6 transects starting with the downstream block net (CHWidth_0), the subsequent transects (CHWidth_5 to CHWidth_20), and ending with the width of the channel at the upstream block net (CHWidth_25).
22. Following Step 21, **Enter** the observed water temperature at each transect into the WTemp fields.
23. Following Step 21, **Enter** the observed water conductivity at each transect into the WCond fields.
24. Following Step 21, **Enter** the observed dissolved oxygen at each transect into the Dissolved Oxygen (DO) fields.

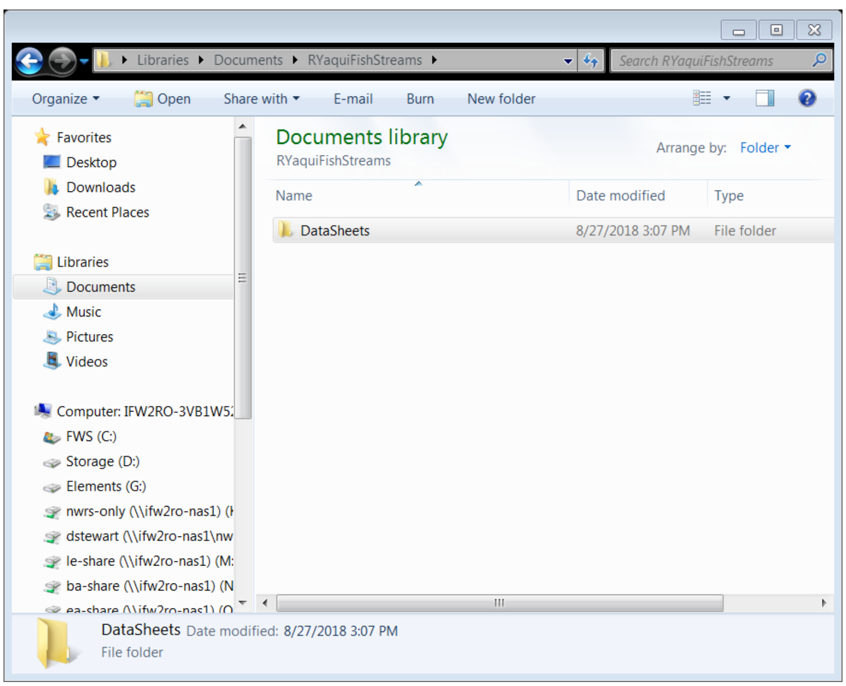
25. Following Step 21, **Enter** the estimated percentage fine substrate at each transect into the SubFine fields.
26. Following Step 21, **Enter** the estimated percentage gravel substrate at each transect into the SubGravel fields.
27. Following Step 21, **Enter** the estimated percentage cobble substrate at each transect into the SubCobble fields.
28. Following Step 21, **Enter** the estimated percentage Boulder substrate at each transect into the SubBoulder fields.
29. Following Figure 3, **Enter** the measured stream velocity for each of the four measurements (identified as the second number ranging from 1 to 4) at each of the 6 transects into the StVelocity fields identified by the first numerical value that ranges from 0 (downstream block net) to 25 (upstream block net).

    Note: *The first and second number identifies the transect (0 to 6) and the measurement (0 to 4). For example, StVelocity_0_1 is the first stream velocity measurement (1) observed at the downstream block net (i.e., 0). Another example, StVelocity_5_2 is the second (2) stream velocity measurement observed at the first 5 meter transect upstream of the downstream block net.*

30. Following Step 29, **Enter** the measured water depth for each of the four measurements (identified as the second number ranging from 1 to 4) at each of the 6 transects into the WDepth fields identified by the first numerical value that ranges from 0 (downstream block net) to 25 (upstream block net).
31. **Initial** and **Date** each data sheet once both species and habitat data have been entered and submitted to Survey123 for each stream reach.
32. **Select** the Start button on your Windows computer
33. **Select** Documents, and then **Select** New Folder
34. **Enter** RYaquiFishStreams as the desired folder name



35. Inside the RYaquiFishStreams folder, **create** a New folder named DataSheets..

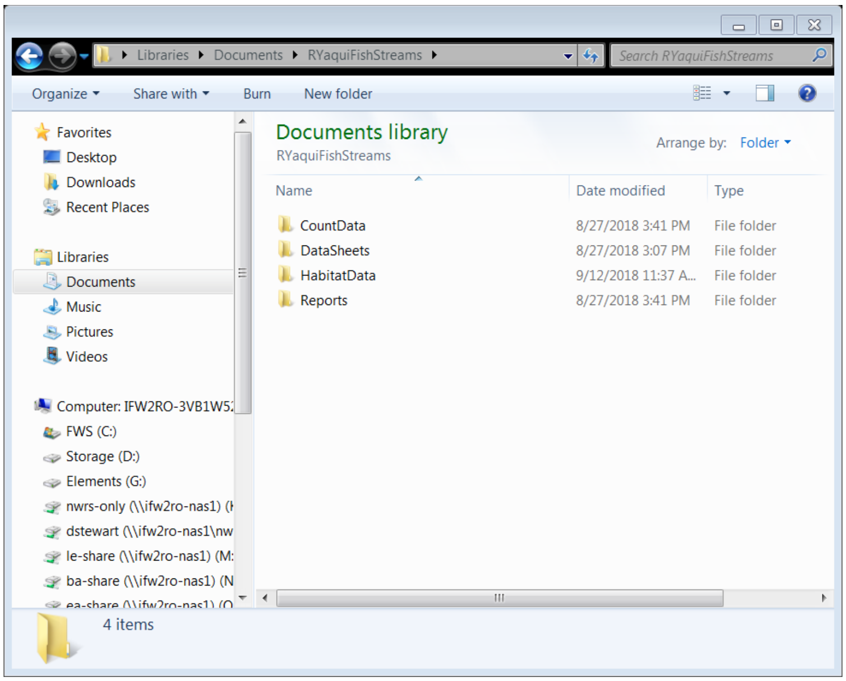


36. **Scan** and **upload** the paper datasheets and save the scans to the RYaquiFishStreams file folder as RYAQUI_stream_yyyy.docx.

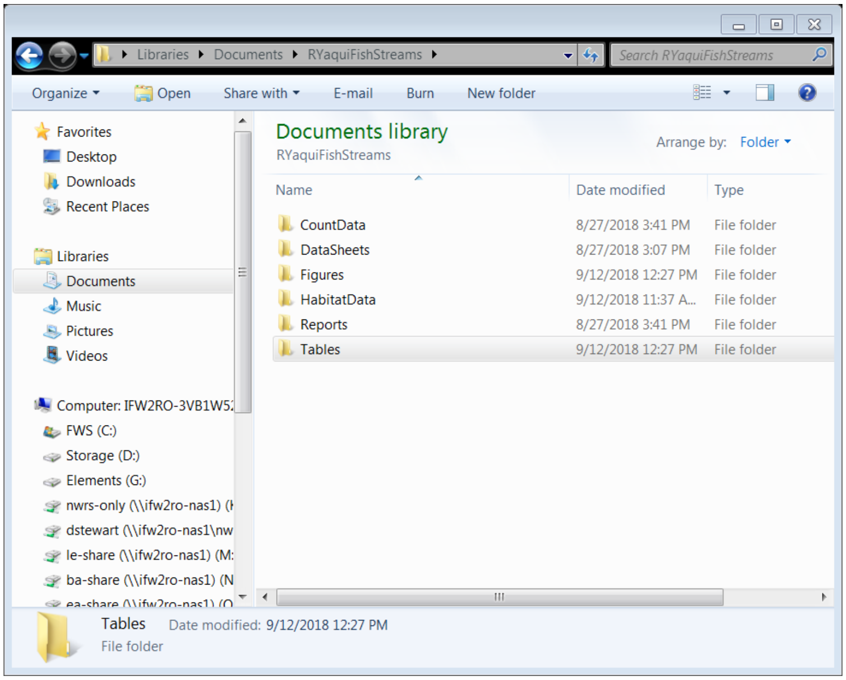
Note: *yyyy indicates the current year of sampling. For example, if the current year is 2018, then save it as 2018.*

37. **Create** count, habitat, and report files. The desired folder names should be
    a. CountData
    b. HabitatData
    c. Reports

Note: *The files labeled CountData and HabitatData will contain the depletion counts and environmental specific information from Survey123. The Reports folder will contain the reports generated for each reporting schedule.*

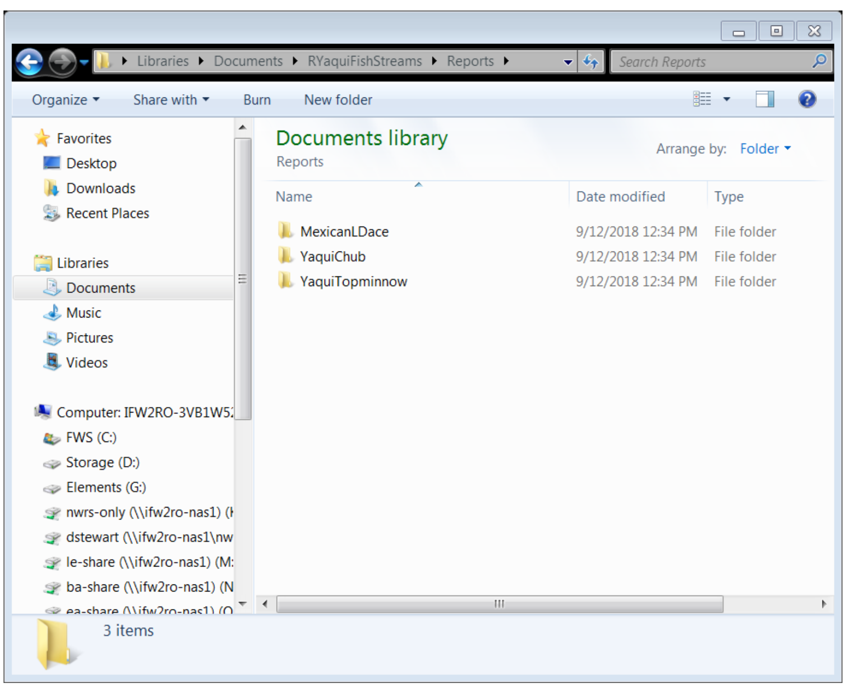


38. **Create** a Figures and Tables file folder.



Note: *The Figures and Tables will contain the Final Results for each species.*

39. **Create** a species file for each of the three species within the Figures and Tables folders.
    a. MexicanLDace
    b. YaquiChub
    c. YaquiTopminnow



Note: *The files labeled as MexicanLDace (Mexican longfin dace), YaquiChub (Yaqui chub), and YaquiTopminnow (Yaqui topminnow) will contain species-specific information.*

40. **Download** the count data file from the Survey123 ArcGIS stream reach and store the file in the working directory created for data pertaining to Río Yaqui fish and streams (i.e., RYaquiFishStreams).
    a. To do this, **Visit** the My Surveys folder located at

       https://survey123.arcgis.com/surveys

    b. Next, S**elect** the Rio Yaqui Fish Depletion Sampling sheet
    c. **Select** the Data tab from the menu
    d. **Select** Export, then select CSV to export the sheet as a csv file

       Note: *The Survey123 sheet will download to your computer as a csv file with its own naming convention. You will need to save it to the CountData folder within RYaquiFishStreams using a different convention (see below).*

    e. **Select** the recently downloaded file (bottom of your screen), and **save** the file to the CountData file folder within the RYaquiFishStreams using the following naming convention count_streamsurvey_yyyy_yyyy.csv

       Note: *Count identifies that the file contains the depletion counts per pass for each species. The first yyyy identifies when the initial year of the survey being implemented (i.e., 2018). The second yyyy identifies the current survey year (e.g., 2019 and so on). For example, if the survey was first implemented in 2018 and the current survey year is 2020, the file should be named count_streamsurvey_2018_2020.csv.*

41. Next, **Download** the habitat data file from the Survey123 ArcGIS stream reach and the file to the working directory created for folder identified for data pertaining to Río Yaqui fish and streams (i.e., RYaquiFishStreams).
    a. To do this, **Visit** the My Surveys folder located at

       https://survey123.arcgis.com/surveys

    b. Next, S**elect** the Rio Yaqui Fish Habitat Data survey
    c. **Select** the Data tab from the menu
    d. **Select** Export, then select CSV to export the sheet as a csv file
    e. **Select** the recently downloaded file, and save the csv sheet to the HabitatData file folder within the RYaquiFishStreams using the following naming convention habitat_streamsurvey_yyyy_yyyy.csv

Note: *Habitat identifies that the file contains the environmental covariates measured at each stream reach. The first yyyy identifies when the initial year of the survey being implemented (i.e., 2018). The second yyyy identifies the current survey year (e.g., 2019 and so on). For example, if the survey was first implemented in 2018 and the current survey year is 2020, the file should be named hab_streamsurvey_2018_2020.csv.*

42. **Verify** that the data are correct for accuracy for each species and habitat data, and then contact the Regional Statistician for analysis.
43. **Complete** the metadata (Element 5).

SOP 3: Data Preparation for Hierarchical Multi-season Depletion Analyses using JAGS

UNDERSTANING THIS DOCUMENT

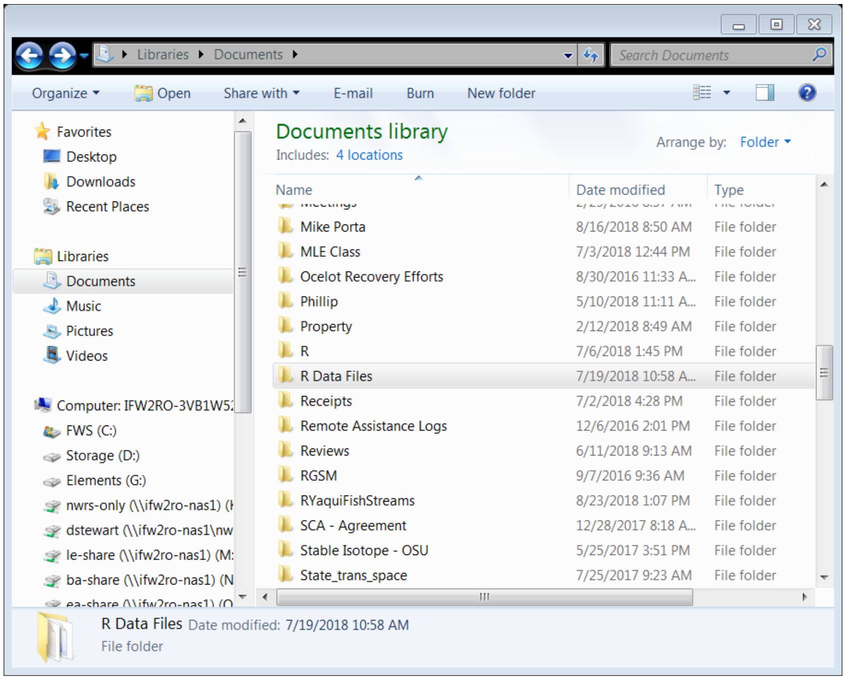
- Emboldened terms are commands, tools, or tasks within the referenced software programs (i.e., Microsoft Excel 2010, Program R).
- Italicized text indicates background information, a filename, or a field name.
- Text in `Lucidia Console` font indicates it is a function, package, library, or a directly executable command line (i.e,. can be copied and paste into the command prompt in the software) in Program R.
- SOP written for a Windows 10 environment.

Consult Regional Statistician to determine if the Refuge staff will complete this SOP, or if the Regional Office I&M staff will complete this SOP. Regardless, NWR staff will complete SOP 4 for data archiving.

Formatting data for hierarchical analysis in Program R.

- Two data frames are needed to conduct Hierarchical depletion sampling analyses in Program R
    1. Count data frame: describes the abundance-pass sampling history from surveyed stream reaches and primary sampling frames among years for each species.
    2. Habitat data frame: describes the habitat conditions from surveyed stream reaches and primary sampling frames among years.

- Information on setting your working directory in Program R
    3. Next, **select** the Start button on your Windows computer
    4. **Select** Documents, and then **Select** New Folder
    5. **Enter** RDataFiles as the desired folder name



a. This folder will be used as your R working directory.
b. It will only include the most "current" count and habitat data, R files, and your workspace will be saved to this folder.

__Note__: Delete all files from previous years prior to copy and paste current years of data into the folder.

6. Once the historical data is **removed** from RDataFiles, **copy** and **save** the most current count (count_streamsurvey_yyyy_yyyy.csv) and habitat (habitat_streamsurvey_yyyy_yyyy.csv) files from RYaquiFishStreams that was previously downloaded from the online Survey123 ArcGIS repository (SOP 2) to the RDataFiles folder.

7. Installing and loading the R packages needed for data analyses in Program R
   1. If necessary **download** and **install** Program R and potentially a user interface to R like R Studio

      https://www.r-project.org/

      https://www.rstudio.com/

   2. If you have not already, **download** and **install** JAGS as per operating requirements

      http://mcmc-jags.sourceforge.net/

   3. **Open** R Studio and **install** R package `jagsUI`

```
install.packages("jagsUI")
```

4. **Load** the package for use in Program R

```
library(jagsUI)
```

5. **Open** R Studio and **install** R package `dplyr`

```
install.packages("dplyr")
```

6. **Load** the package for use in Program R

```
library(dplyr)
```

7. **Open** R Studio and **install** R package `ggplot2`

```
install.packages("ggplot2")
```

8. **Load** the package for use in Program R

```
library(ggplot2)
```

9. If you have not already, **install** package `devtools`

```
install.packages("devtools")
```

10. **Load** the package for use in Program R

```
library(devtools)
```

11. **Install** the `depletion` package from drstewart11 GitHub account for use in Program R

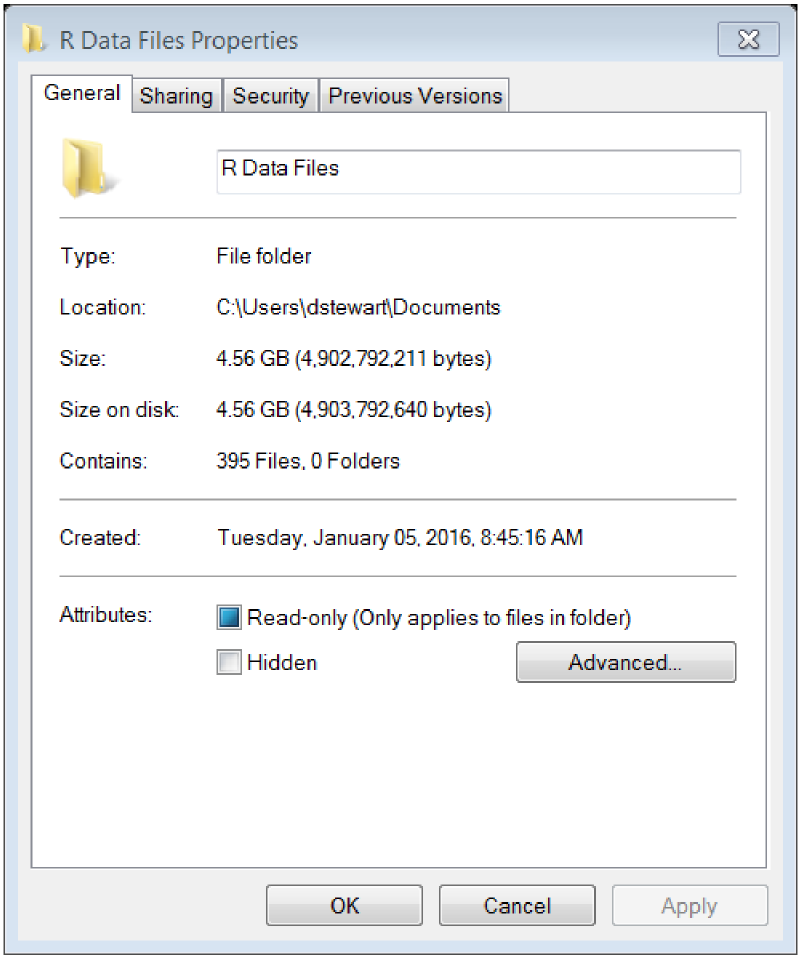
```
install_github("drstewart11/depletion")
```

12. **Load** the package for use in Program R

```
library(depletion)
```

Note: *If these packages were previously installed, then use* `library` *to* **load** *each the package for use in Program R (e.g., library(depletion)).*

8. Import the count_streamsurvey_yyyy_yyyy.csv and habitat_streamsurvey_yyyy_yyyy.csv files into Program R
   1. **Open** Program R using the RStudio interface.
   2. **Set** the working directory.
      a. To do this, first right **Click** on folder name RDataFiles from the Documents list.
      b. **Select** Properties.



c. The directory path will be identified by the Location field
   i. C:/Users/……/Documents
   ii. The "…" in the directory path above is the computer name, which varies by machine and network. Do not use ….. in the directory path.
d. To set the working directory in Program R. **Type**

```
setwd("C:/Users/[insert remaining file
name]/Documents/RDataFiles")
```

3. **Load** the Count and Habitat datafiles (i.e.,) and **name** them as follows

```
countdat=read.csv("count_streamsurvey_yyyy_yyyy.csv.csv",heade
r=T,sep=",",na.strings="")

Hab=read.csv("habitat_streamsurvey_yyyy_yyyy.csv.csv",header=T
,sep=",",na.strings="")
```

9. Information on "`depletion`"
1. Abundance data array's YChub, YTop, and MDace (count): describes the abundance histories for each pass, stream reach, and year of when a

"`depletion`" survey was completed. Internally, function `depletion` within "`depletion`" will sort and reorganize the data by species.

2. Habitat data array (Hab): describes the physical habitat data measured at each stream reach and year. This file will also include the number of seconds per pass, electroshocking settings, and number of netters for each stream reach and year.
3. The model within function `deplete` is defined in JAGS syntax, which is similar to that of WinBUGS and OpenBUGS.
4. The data downloaded from the ArcGIS folder is prepared as inputs for the jags function.
5. Internally the species-specific count data (count) and habitat data (hab) is being ordered, sorted, and packaged into separate arrays for analysis.
6. Once the data is packaged, the JAGS model will initialize. It may take a few minutes to hours to complete the Markov chain Monte Carlo chains to complete. You will see progress in the R console.
7. R function "`depletion`" will produce two sets of results.
    a. A Table that contains stream reach- and year-specific abundance estimates and their associated error rates (i.e., 95% credibility intervals).
    b. A Figure illustrating the results depicted in the Table.
8. "`depletion`" will automatically save the Table and Figure to your working directory (i.e., RDataFiles). For example, if you identify species="YChub" in the deplete function (see below), then the Table will be saved as a .csv file named "YaquiChubStreamResults" and the Figure will be saved as a .tiff file named "YaquiChubStreamPlot".

    ***Note**: The results will be saved as "YaquiTopminnowStreamResults" and "YaquiTopminnowStreamPlot" when "YTop" is identified in the function, and "MexicanLDaceStreamResults" and "MexicanLDaceStreamPlot" when "MDace" is identified.*

***Note**: Both 7a and 7b are needed for reporting.*

10. Information on the depletion model
    1. Though the R code to produce the results can be found online, once package "`depletion`" is loaded into the R environment using `library`, then the only R syntax needed to run the model is

    ```
    deplete(count=countdat,hab=Hab,species=c("YChub","YTop","MDace
    ")
    ```

    2. Select the species of interest (e.g., YChub) from the species list. Selecting more than one species name will result in an error. For example,

    ```
    deplete(count=countdat,hab=Hab,species="YChub")
    ```

3. Internally the data for Yaqui Chub is being packaged (sorted, rearranged, and inserted into an array) for analyses.

4. Once the data is packaged and identified in the `deplete(count=countdat,hab=hab,species="YChub")` function, then `jagsUI` is used to call JAGS from R to run the Hierarchical Bayesian Depletion Model. The model will initiate, iterations are completed internally, and results are immediately saved to your working directory, where the following functions are called to run the Bayesian model

   #R code: `jagsUI` example
   #Bayesian Data Analysis

   ```
   jags.data <- list(count=dataCount, totalC=Nst, list of habitat
   and sampling data variables, nsite=dim(dataCount)[1],
   nrep=dim(dataCount)[2],nyear=dim(dataCount)[3])
   ```

   #dataCount = reordered and sorted species-specific count data
   #Nst = total number of fish captured at each stream reach and year
   #nsite = total number of stream reaches surveyed on an annual basis
   #nyear = total number of years that a stream reach was repeatedly sampled
   #nrep = total number of successive depletion passes

   ```
   jags.params <- c()
   ```

   #`jags.params` specifies a list of parameters to save during the model iterations.

   ```
   jags.inits<-function(){
   list()
   }
   ```

   #`jags.inits` provides initial values for each parameter. These random initial values are specified internally.

   ```
   jagsfit<-
   jags(data=jags.data,inits=jags.inits,jags.params,n.iter = ,
   n.chains = ,
   n.thin = , n.burnin = , model.file=depletion.file)
   ```

   #`jagsfit` is a function that is used to package the data, initial values, parameters to save, iteration description, and model file to JAGS from R

   ```
   print(jagsfit)
   ```

   #`print` displays the output

   ***Note**: Depending on the species identified in the function, the Table and Figure will be automatically saved to your RDataFiles working directory.*

5. **Repeat** for these steps for the remaining two species.

11. Saving the Data, Figures and Results
    1. **Return** to the RDataFiles file folder.

       Note: *You will notice that both Tables and Figures were created for each species. You must open and save these to the species-specific Tables and Figures folders within RYaquiFishStreams before deleting*

    2. **Select** a Table within RDataFiles and **save** the Table as a .csv file *species_table_yyyy_yyyy* in the species-specific Tables file folder located in RYaquiFishStreams
    3. **Repeat** for the remaining species
    4. **Select** a Figure within RDataFiles and **save** the Figure as a .tiff file using the naming convention *species_figure_yyyy_yyyy* in the species-specific Figures file folder located in RYaquiFishStreams

       Note*: The species-specific file naming convention relies on the acronyms YChub, YTop, or MDace to identify the species. The first yyyy identifies the first year of the survey (i.e., 2018). The second yyyy identifies the most current year of the survey*.

    5. After saving the files to their new location in RYaquiFishStreams, **delete** all files from the RDataFiles file folder. It should be empty.
    6. **Repeat** SOP 3 until the analysis is completed for all fish species.

SOP 4: Data Archiving

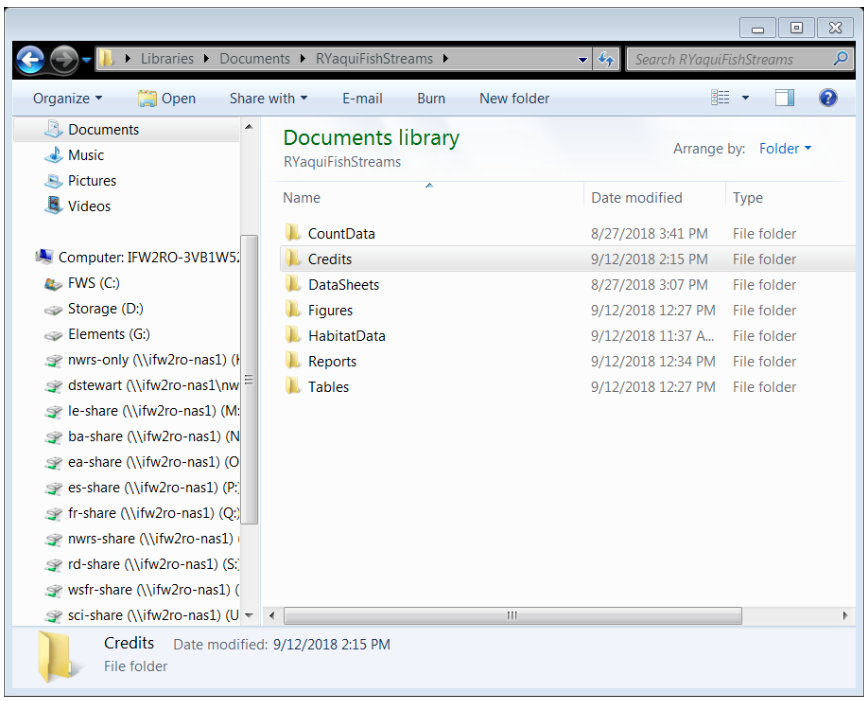
UNDERSTANING THIS DOCUMENT

- Emboldened terms are commands, tools, or tasks within the referenced software programs (i.e., Microsoft Excel 2010, Program R).
- Italicized text indicates background information, a filename, or a field name.
- Text in `Lucidia Console` font indicates it is a function, package, library, or a directly executable command line (i.e,. can be copied and paste into the command prompt in the software) in Program R.
- SOP written for a Windows 10 environment.

Archiving and backup of survey data

Once SOPs 1-3 are completed for the daily surveys, the data will be copied over to the RioYaquiFish Stream Surveys ServCat site to provide an off-site data storage location in case a catastrophic event occurs such as server failure or hard-drive failure.

1. If the following steps 2-5 were completed previously, then **skip** to step 6.
2. After completing SOP's 2-3, **create** a subfolder in the RYaquiFishStreams folder and title it **Credits**

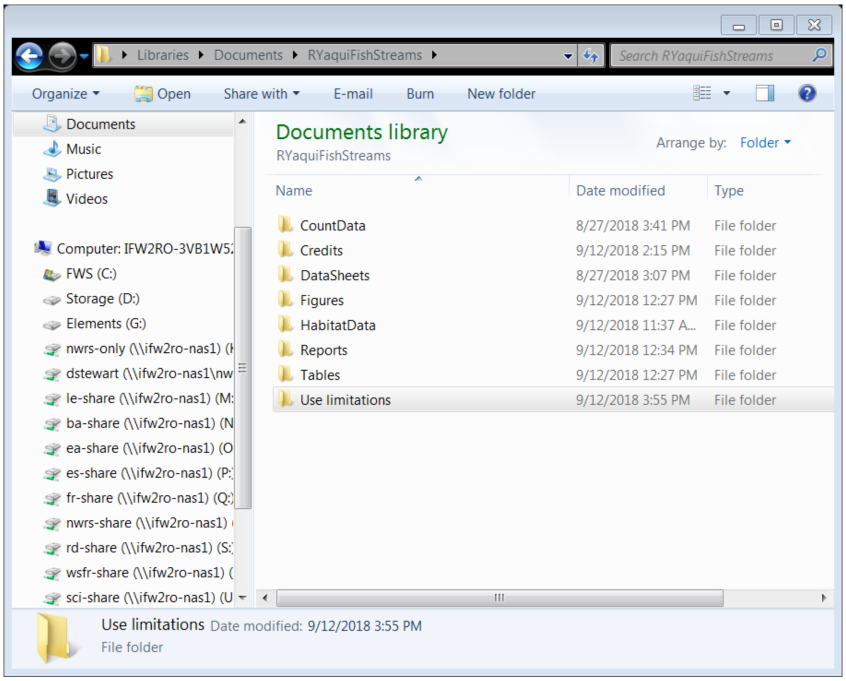


3. And then **upload** a word document that reads:

   "These objects were created following the survey protocol for Rio Yaqui fish in streams for San Bernardino NWR (Version 1.1).

Stewart DR, Johnson, LA, Eichhorn C. 2018. Survey protocol framework for monitoring abundance of Río Yaqui fish in streams: San Bernardino and Leslie Canyon National Wildlife Refuges. Version 1.1. Survey Identification Number: FF02RASB00-059. U.S. Fish and Wildlife Service, Douglas, Arizona, USA."

4. **Create** a subfolder entitled **Use limitations** in the RYaquiFishStreams folder



5. And then **upload** a word document that reads:

   "For official use only by San Bernardino NWR staff to complete the "Survey Protocol Framework for Monitoring Abundance of Rio Yaqui Fishes in Streams (Version 1.1)." Not for any other use. The USFWS is not responsible for any other use, or misuse of this dataset. Any use of this dataset and R script identified in the Appendix (or on GitHub) should reference the protocol.

   Note: *The word documents related to Credit and Use limitations need to be uploaded to the Credit and Use limitation folders in ServCat, but only once. These need to be revised with each revision of the Survey Protocol.*

6. To upload the file(s) to ServCat, one will first need to **create** a Compressed (zipped) folder by right clicking on the folder RYaquiFishStreams, then selecting "Send to", and clicking Compressed (zipped) folder. The naming convention of the file is similar to the above convention where the RYaquiFishStreams folder will identify the year the Survey started to the

current survey year, as RYaquiFishStreams_yyyy_yyyy so that those working with the data set understand the range of dates that the data history captures.

***Note**: The first yyyy identifies the first year of the survey (i.e., 2018). The second yyyy identifies the most current year of the survey. For example, if this survey started in 2018 and the current survey year is 2020, then the file will be saved as RYaquiFishStreams_2018_2020.*

7. Next, **visit** the ServCat project folder at

   https://ecos.fws.gov/ServCat/Reference/Profile/104820

8. **Select** the Actions drop-down tab, and then **Select** Edit.
9. **Select** Files and Links.
10. **Select** the drop-down tab Add.
11. **Select** Add Digital File.
12. **Select** Browse, navigate to the newly created Compressed (zipped) folder, and then select the file.

    ***Note:** Please identify that the file being uploaded is the one that corresponds to the most current survey year.*

13. In the Description field, please provide a brief description of the data and any issues that may surround this data so that others are aware of complications prior to downloading the data.
14. **Select** Add once the Description field is completed.
15. Next, **Select** Activate to **Save** & **Close** the Project.

Appendix A: Issues with the traditional approach.

From a far the traditional sampling approach appeared to be correct and align with other sampling approaches used to inventory and monitor fish in lotic environments. Because the approach accounts for detection probability, the utility of the approach allowed for us to make slight improvements to help improve the reliability of the information being produced for each of these species across all sampling stream reaches and through time, as well ensure continuity in the implementation of the survey in the event of staff turnover.

First, at both San Bernardino NWR and El Coronado Ranch (West Turkey Creek, AZ), we identified a common problem that also persists among many programs that rely on backpack electroshocking devices, where the methods to adjust the voltage and duty cycle settings of the Smith-Root back pack electroshocking unit are not clear, and thus the settings of the unit were not adjusted according to the conductivity of the stream and amperage output. It is important that the equipment needs to be tailored to the environmental conditions to further standardize effort so that changes in catch are reflective of the population and how it relates to the environment instead of how catch may be affected by the ineffectiveness of the sampling equipment due to sampling conditions. The method to implement these changes may vary with the experience of biologist and their familiarity with the electroshocking backpack unit. Therefore, in this protocol it is procedures are outlined to change these settings for each stream reach prior to the first depletion pass. Moreover, and historically, the habitat conditions at the time of sampling were never considered to be important to measure. It was not until the Refuge measured habitat that we identified several variables that not only helped describe their ecology but why they were not able to capture these species in some habitats compared to others. Refuges now knows from this pilot work that stream geometry, channel unit, stream flow and depth as negatively influencing capture ability of each species (Stewart et al. 2019 or Element 2 (Appendix B)). If one does not measure and include these effects in the model, then one risk greatly underestimating abundance of each of these species at a stream reach.

Second, many technological and statistical advances have resulted in improvements in data collection and analysis techniques since the depletion experiments began in 2004. Refuges sought to update the existing approach so that it is current with these methods. Moreover, Refuges identified a series of objectives that directly relate to the Río Yaqui fish stream monitoring program that are clearly articulated in this protocol, while also seeking to provide detailed documentation and standardization to ensure repeatability of future efforts in the event of staff turnover. Though a database was previously developed to house stream sampling data, Refuges sought to develop one that could store the data online, on ServCat (online repository), as well as be read into R statistical program to generate Tables and Figures for the annual and five year reports. In doing so, it takes the existing approach and helps strengthen it by creating a set of steps that will allow the generation of results to be logistically easier and will help with reporting procedures.

Third, at San Bernardino NWR, the former approach does not describe the importance of visually inspecting block nets (i.e., feeling the bottom of the net or using the dip net to collect individuals after the electroshocking pass that may have become entangled or are pushed into the folds of the net, ect.) before, during, and even after sampling. Visually inspecting block nets throughout the survey period is necessary to ensure that one maintains a closed population; otherwise, fish may leave the enclosed area by swimming under, around, or over the block nets. Fourth, fish may respond to sampling by swimming into the net to avoid capture to only return to the sampling unit after the depletion pass has concluded. This is another often overlooked step because in not doing so the abundance estimate produced is biased and unreliable, thus after each electroshocking pass crewmembers will be required to sample and collect any and all individuals near the net. Fifth, though effort (i.e., number of seconds per pass) should remain constant across all passes, the amount of effort based on historical data indicates that effort varied among each of the three passes. For example, based on the historical data, the first pass was slower (e.g., 321 seconds) on average than the remaining two successive passes (e.g., 180 seconds). Together (3-5) these effects violate the assumptions of the sampling technique and may lead to significant variation in detection probability across successive passes (Stewart et al. 2019 and Element 2).

Sixth, at El Coronado Ranch (West Turkey Creek, AZ), the survey approach differs from the one used to survey this species at San Bernardino NWR. For example, the sampling design consisted of sampling a 100-m stretch of river using a single pass. The upper and lower boundaries of the stream reach were not defined with block nets to establish a closed population or prevent fish escapement. Simple count is used instead of a population estimate produced from unmarked methods, and thus this approach relies on a species-specific index of abundance to inform management. Therefore, Refuges identified and implemented a few alternative approaches which borrow from the strengths of the traditional method used at San Bernardino NWR to help with standardization of methods used to survey these streams reaches, such as identifying steps to establish a closed population (to ensure that no individuals are immigrating/emigrating from the sampled area), identified a series of procedures to implement when starting and completing a depletion pass, and also developed a statistical model to produce a “true” abundance estimate corrected for detection probability for all stream reaches and locations. Additionally, this will now require the Refuge to survey four 25 m stretches of river within the 100 m section using block nets. This modification makes surveying these stretches of river more manageable during the survey year, removes uncertainty related to which method to use, and now the survey will be able to track a meaningful abundance estimate for each of these species through time.

We again applaud the traditional approach that considered detection probability to estimate a “true” abundance estimate. It is already well known that using an index of abundance like CPUE is problematic and can’t be used to reliably assess status of a species in streams or inform recovery because of changes in annual count may not be reflective of anything more than changes in capture efficiency (Stewart et al. 2017a). The strengths of the traditional method established a foundation to continue advancing the biological program at San Bernardino NWR. In this protocol, Refuges identify the stream reach-specific strengths of the traditional approach at San Bernardino NWR and El Coronado Ranch, while also identify how we improved and also added to as a way to strengthen the monitoring of Río Yaqui fishes in streams at and around the Refuge. Simple approaches are outlined to address these minor issues in this protocol.

Appendix B: Stewart et al. 2019 – Efficacy of depletion models for estimating abundance of endangered fishes in streams.

https://www.sciencedirect.com/science/article/pii/S0165783618302674

Appendix C: Data Sheet.

Stream reach ID:		Date:	
Easting:	Northing:	UTM Zone:	

Stream subsystem (circle): Perennial Intermittent	Backpack electroshocking settings		
	Frequency	Duty cycle	Voltage

Field measurements	Wood pieces:	Number of nets:

	Channel characteristics								Substrate characteristics[2]			
Interval (m)	Channel unit[1] (Riffle=1, Run=2, Pool=3)	Channel width (cm)	pH	W. temp	D.O.	Sp. conduct	NTU	Algal	Fine	Gravel	Cobble	Boulder
0 (lower net)												
5												
10												
15												
20												
25 (upper net)												

Stream velocity/depth along four transects								
	Stream velocity (m/s)				Water depth (cm)			
	Transect				Transect			
Interval (m)	1	2	3	4	1	2	3	4
0 (lower net)								
5								
10								
15								
20								
25 (upper net)								

[1]Riffle = shallow-fast flowing water; Run = shallow to deep water; Pool = deep water

[2]Fine = < 5mm; Gravel = 5-50 mm; Cobble = 50-300 mm; Boulder = >300 mm

Appendix C: continued.

Species acronyms and common names: GIPU – Yaqui chub, POSO – Yaqui topminnow, AGCH – Mexican longfin dace

Depletion pass[3]	Seconds per pass[4]	GIPU	POSO	AGCH
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

[3]Insert NA for each pass following the stopping point of the survey

[4]Number of seconds per pass

Appendix D: Example 2017 State of Arizona Game and Fish Department Scientific Collection Permit.

State of Arizona
Game and Fish Department
No refunds/Not transferable

LICENSE YEAR: 2017
HQ - PHOENIX
FREE E-NEWS SIGNUP
www.azgfd.gov/signup

DATE:
RESIDENT: [redacted]

LIC [redacted] SCIENTIFIC COLLECTING VALID: [redacted]

[redacted]

BIRTH-DATE HEIGHT WEIGHT EYES HAIR SEX

[redacted]

PO BOX 3509
DOUGLAS, AZ 85608

I hereby certify all information on this license is true.

NOT VALID UNTIL SIGNED

SCIENTIFIC COLLECTING LICENSE STIPULATIONS

WILLIAM R. RADKE ▪ USFWS, BUENO AIRES NATIONAL WILDLIFE REFUGE COMPLEX

1. The following are agents under this license for the activities below:

Geoff Bender	Sharon Glock	Paula O'Brian
Clark Bloom	Jason Greff	Adrien Radke
Aaron Cajero	Tasha Harden	Marcia Radke
Rebecca Chester	Victor Harden	Shannon Radke
Justin Congdon	Lacrecia Johnson	Humberto Rodriguez
Nancy Congdon	Brenda Leon	Katie Schober
Stan Culling	Anna Magoffin	Joshua Smith
Fred Dunn	Matthew Magoffin	Anne Steffler
Vicki Dunn	Chuck Minckley	Randy (David) Stewart
Charles Glock	Robert Minckley	

The licensee OR the agent(s) MUST be present at all activities conducted under authority of this license and must have a copy of the license and stipulations present at all times while conducting activities.

2. This license allows stipulated activities to be conducted: **Statewide.**

3. You must notify by email to the appropriate Aquatic Wildlife Program Managers, Specialists and Coordinators (see list that follows) prior to field collections and sampling. We recognize that you have regularly scheduled monitoring efforts and would like to better coordinate to reduce duplication of effort (by us or other investigators), better respond to public and law enforcement inquiries on activities that might be perceived by them as illegal, and to assist other investigators in acquiring needed specimens for propagation and research.

Invertebrates Program Manager: Jeff Sorensen (jsorensen@azgfd.gov; 623-236-7740)
Statewide Native Aquatics Program Manager: Julie Carter (jcarter@azgfd.gov; 623-236-7576)
Native Aquatic Specialist (Topminnow/Pupfish): Ross Timmons (rtimmons@azgfd.gov; 623-236-7509)
Region V Aquatic Program Manager: Don Mitchell (dmitchell@azgfd.gov ; 520-388-4451)

4. You are authorized unlimited capture and release mollusk/crustacean species (capture of federally listed species requires a federal permit).

5. You may collect and kill unlimited numbers of crayfish.

6. You are authorized to capture and release unlimited numbers of native and non-native fish in coordination with the appropriate species leads and Regional Program Manager listed in stipulation #3; Non-target species must

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Appendix D: continued.

be released alive; removal of non-native fish is on a case-by-case basis and must be coordinated with the Regional Program Manager.

7. You are authorized to capture by hand or hand-held implement, hold and release at the site of capture, unlimited numbers of amphibians and reptiles (both open and closed season species). Survey for or capture of federally listed species requires a federal permit. Any activities involving Sonoran tiger salamanders (*Ambystoma tigrinum stebbinsi*) must be coordinated with **Thomas R. Jones 623-236-7735 or tjones@azgfd.gov** *email preferred.*

8. You are authorized to survey for Chiricahua leopard frog (*Rana chiricahuensis*), including capture, photograph and release immediately at site of capture.
   a. You must coordinate your efforts with AGFD Ranid Frogs Project staff (see below).
      RU 1, 2, 3, & 4; **Hunter McCall (hmccall@azgfd.gov, 623-236-7378)**
      RU 5, 6, & 7; **Cody Mosley (cmosley@azgfd.gov, 623-236-7189)**
   b. You and your agents are authorized to survey for Chiricahua leopard frogs **only** 1) if all have attended the Chiricahua leopard frog certification workshop within the last 4 years, or 2) have had 40 hr. or more of field training with a qualified person approved by AGFD and USFWS, or 3) if it has been more than 4 years since attending the workshop but you and your agents continually survey for Chiricahua leopard frogs.
   c. All new Chiricahua leopard frog localities (i.e., previously unknown sites or sites that have not been occupied for 5 years or more), and dead Chiricahua leopard frogs or die-offs must be reported as soon as possible, but no later than 5 business days of discovery to **AGFD Ranid Frogs Project Coordinator, Audrey Owens (aowens@azgfd.gov, 623-236-7515)**; Refer to the Department's *Amphibian Die-off Protocol* for collection of any dead specimens.
   d. You must submit Protocol survey forms for all Chiricahua leopard frog surveys (both positive and negative detects) to AGFD Ranid Frogs Project staff by **December 1st** of that survey year.
   e. Use the Department *Field Work Disease Prevention Protocol* to disinfect and sanitize following activities.

9. When requested to do so by AGFD and/or USFWS for Chiricahua leopard frogs, you are authorized to capture, hold, collect eggs, larvae, or frogs from donor sites to be used for translocation, transport, release, and headstarting. Requests may take the form of email correspondence or hard copy letter.

10. You are authorized to capture, collect toe clips, and release frogs, including Chiricahua leopard frogs (*Rana chiricahuensis*), for identification and Bd testing. Please coordinate your efforts with **AGFD Ranid Frogs Project Coordinator, Audrey Owens (aowens@azgfd.gov, 623-236-7515).**

11. You may collect up to five (5) per species of open-season amphibians and reptiles.

12. You are authorized to kill unlimited numbers of bullfrogs (*Rana catesbeiana*), Rio Grande leopard frogs (*Rana berlandieri*), carp (*Cyprinus carpio*), goldfish (genus *Carrasius*), mosquitofish (genus *Gambusia*), green sunfish (*Lepomis cyanellus*), bullheads (genus *Ameiurus*), crayfish and nonnative snails in habitats where they threaten native aquatic wildlife. Where appropriate and safe you may kill bullfrogs with pneumatic weapons or .22 rifle.

13. You **must** coordinate all work prior to activities on Mexican gartersnakes (*Thamnophis eques*) or narrow-headed gartersnakes (*T. rufipunctatus*) with the **AGFD Amphibians and Reptile Program Manager, Tom Jones (tjones@azgfd.gov; 623-236-7735)** *email preferred.*
    a. When using funnel-type traps (e.g., Gee Minnow traps, Promar® minnow traps, hoop nets) to survey in habitat occupied by Mexican gartersnakes, use only 1/8 inch mesh traps. Traps should be set with a portion of the trap above the water so that any captured snakes will be able to breathe.
    b. When surveying for gartersnakes, check traps at least twice a day (am and pm) or more often after deployment until traps are removed from the site.
    c. All unattended nets/traps must be labeled with the Scientific Collecting Licensee's name, SP license number, and contact information.
    d. Any snakes that dies, as a result of sampling must be collected and turned over to the Gartersnake Projects Coordinator.

Appendix D: continued.

e. Any efforts that specifically target Mexican gartersnakes (*Thamnophis eques*) require a federal license.
f. All new localities (i.e., previously unknown sites or sites that have not been occupied for 5 years or more), and dead or die-offs must be reported to AGFD Amphibians and Reptile Program Manager within 5 business days of discovery.
g. Survey for or capture of federally listed species requires a permit from the U.S. Fish and Wildlife Service.

14. You are authorized to capture, band, and release all birds (including raptors) as requested and in accordance with Federal and Migratory Bird Banding Permits (Excluding eagles and threatened and endangered species without an approved project requiring such activities. Federal permits are required for listed species).

15. You are authorized to conduct vocalization playback surveys for Southwestern Willow Flycatcher (*Empidonax traillii extimus*) and Yellow-billed Cuckoo (*Coccyzus americanus*) in accordance with USFWS permit guidelines. **Please note: all agents working under this license must also have successfully completed the USFWS approved training workshop.**

16. You are authorized to capture, mark, photograph unlimited numbers of small nongame mammals using baited box-style animal traps and release immediately within 5 meters at the site of capture. Capture of federally listed or protected species requires a federal permit.

17. You may collect up to five (5) per species of small mammals (take amount for bats listed in stipulations below). Capture of federally listed or protected species also requires a federal permit.

18. Bats may be captured using mist nets or by hand (including hand held implements) and released alive. Bats may NOT be marked using rings, bands, collars, brands, or any other technique. However, bats may be fitted with radio-transmitters and tracked to their roosts.
    a. Bats must be released alive at their capture location within four (4) hours of capture.
    b. Individuals who die as a result of trapping, handling, or marking may be salvaged and must be noted on your year-end report and deposited in an accredited museum.

19. To guard against White-nose Syndrome in the west, we ask that licensees assist with prevention and surveillance of this disease. Please contact Angie McIntire, AZGFD Bat Management Coordinator (623-236-7574; amcintire@azgfd.gov) with questions.
    a. To prevent the spread of WNS, disinfect bat processing equipment between capture sites (i.e., wipe down calipers/ruler, processing table, scales, light box, and other surfaces that will come in contact with bats). Use quaternary disinfectant with a minimum of 0.3% quaternary ammonium compound – 1:128 dilution or 1oz:1 gallon water (e.g. Lysol IC Quaternary Disinfectant Cleaner).
    b. If you travel from the west to visit eastern roost sites, particularly caves and mines, take disposable clothing, footwear, and gear that you can discard in the east before returning west to avoid potential transportation of contaminants. Also, avoid contamination of your vehicle by changing out of clothes used in eastern sites and disposing of or sealing them prior to getting in your vehicle.
    c. Do not enter caves or mines with gear or clothing from a WNS affected area. When entering sites that are used or have the potential to be used as hibernacula: to facilitate footwear decontamination, use rubber boots; remove all soil and organic material from boots, clothing and equipment; wash all clothing in hot cycle and dry in dryer; and rinse and disinfect footwear using quaternary ammonium compound and air dry.

20. To prevent the spread of WNS, individuals who frequent bat-roosting habitat need to be aware of the symptoms. The following unusual appearances or behaviors may be signs of WNS:
    a. White fungus, especially o the bat's nose, but also on the wings, ears, or tail
    b. Emaciated or dehydrated bats leaving hibernacula
    c. Bats flying outside during the day in temperatures at or below freezing
    d. Bats clustered near the entrance of hibernacula
    e. Dead or dying bats on the ground or on buildings, trees or other structures

Appendix D: continued.

21. For the following bat species up to 5 individuals may be collected per location and transported dead out of the State. No more than 20 individuals per species may be collected.

California myotis (*Myotis californicus*)
Arizona myotis (*Myotis lucifugus occultus*)
W. small-footed myotis (*Myotis ciliolabrum*)
Big brown bat (*Eptesicus fuscus*)
Silver-haired bat (*Lasionycteris noctivagans*)
Mexican free-tailed bat (*Tadarida brasiliensis*)
Yuma myotis (*Myotis yumanensis*)
Long-legged myotis (*Myotis volans*)
Cave myotis (*Myotis velifer*)
W. pipistrelle (*Pipistrellus hesperus*)
Hoary bat (*Lasiurus cinereus*)
Pallid bat (*Antrozous pallidus*)

22. For the following bat species up to 5 individuals may be collected per location and transported dead out of the State. No more than 10 individuals per species may be collected.

Southwestern myotis (*Myotis auriculus*)
Long-eared myotis (*Myotis evotis*)
Fringed myotis (*Myotis thysanodes*)
Big free-tail bat (*Nyctinomops macrotis*)
Pocketed free-tail (*Nyctinomops femorosaccus*)
W. mastiff bat (*Eumops perotis*)
Townsend's big-eared bat (*Corynorhinus townsendii*)
California leaf-nosed bat (*Macrotus californicus*)

23. For the following bat species up to 2 individuals may be collected per location and transported dead out of the State. No more than 5 individuals per species may be collected.

Lesser long-nosed bat (*Leptonycteris curasoae*) -- requires Federal Permit
Mexican long-nosed bat (*Leptonycteris nivalis*) -- requires Federal Permit
Ghost-faced bat (*Mormoops megalophylla*)
Mexican long-tongued bat (*Choeronycteris mexicana*)
Spotted bat (*Euderma maculatum*)
W. red bat (*Lasiurus blossevillii*)
W. yellow bat (*Lasiurus xanthinus*)
Allen's lappet-browed bat (*Idionycteris phyllotis*)
Underwood's mastiff bat (*Eumops underwoodi*)

24. For all nongame mammals including bats: one voucher individual may be taken from each single population/locality of which female bats must be nonparous or post-lactating. Location information for all bat species captured must be reported in the year-end collecting report.

25. Approval of additional coordinated activities may take the form of email correspondence or hard copy letter from Department Program Managers and Wildlife Specialists.

26. You and all agents listed in this license may salvage wildlife found dead (salvage of federally listed species requires a federal permit).

27. The disposition of all wildlife handled or surveyed during activities must be reported on the *SCL Report Form* provided (captured/released alive, collected, fatalities, salvaged, and including positive location from surveys).

Closed-season species are:
For amphibians: Sonora tiger salamander (*Ambystoma tigrinum stebbinsi*) plains leopard frog (*Rana blairi*), Chiricahua leopard frog (*Rana chiricahuensis*), relict leopard frog (*Rana onca*), northern leopard frog (*Rana pipiens*), Tarahumara frog (*Rana tarahumarae*), and lowland leopard frog (*Rana yavapaiensis*).

For reptiles: flat-tailed horned lizard (*Phrynosoma mcallii*), Gila monster (*Heloderma suspectum*), Chuckwallas (*Sauromalus*) from within the boundaries of Phoenix South Mountain Park, shovel nosed snake (*Chionactis occipitalis*) from Pima County east of the Tohono O'odham Indian Reservation or from Pinal County, milk snake (*Lampropeltis triangulum*) in Cochise County, Mexican gartersnake (*Thamnophis eques*), narrow-headed gartersnake (*Thamnophis rufipunctatus*), rock rattlesnake (*Crotalus lepidus*), twin-spotted rattlesnake (*Crotalus pricei*), ridge-nosed rattlesnake (*Crotalus willardi*), massasauga (*Sistrurus catenatus*), ornate box turtle (*Terrapene ornata*), Mojave desert tortoise (*Gopherus agassizii*), and Sonoran desert tortoise (*Gopherus morafkai*).

END

AZGFD ▪ 5000 W. Carefree Hwy ▪ Phoenix, AZ 85086 ▪ 602-942-3000

Appendix D: continued.

Arizona Game and Fish Department
Scientific Collecting License
Standard Notes

A) THIS LICENSE IS VALID FOR OFFICIAL USE ONLY. USE OF THIS LICENSE FOR PERSONAL OR OTHER ACTIVITIES NOT IDENTIFIED IN PROPOSAL IS PROHIBITED.

B) As per A.R.S. Title 17 102 and 306, and Commission Rule R12-4-402, live wildlife, parts thereof, or their progeny, obtained or held under the authority of this license may not be offered for sale, traded, bartered, loaned for the purposes of commercial activity, given as a gift, or disposed of in any way except as stipulated by the Department. Live wildlife, parts thereof, or their progeny, obtained or held under the authority of this license shall remain the property of the State of Arizona. Upon completion of the licensed activity, live wildlife, parts thereof, or their progeny will only be disposed of as per the direction of the Department.

C) This license does not authorize activities with federally listed species nor does it authorize activities on federal or tribal lands unless appropriate federal and tribal licenses are obtained. Additional licenses/permission from the land owner/manager or resource management agency may be required for access and/or collecting on National Park Service, National Wildlife Refuge, National Monuments, Department of Defense, Forest Service, Bureau of Land Management, State Parks, State Monuments, or private lands. In addition, licenses from other states or the federal government may be required when transporting across state lines and holding live wildlife.

D) This license does not authorize activities with any plants, insects, or arachnids.

E) Specimens, including incidental take and salvage, whose collection was intended primarily for scientific study, must be deposited in a United States museum that is accredited by either the American Society of Mammalogists (spreadsheet titled, "Mammal Collections in the Western Hemisphere, updated 2012" electronically accessible at http://www.mammalsociety.org/committees/systematic-collections#tab3) or listed by the American Society of Ichthyologists and Herpetologists (Sabaj Pérez, M.H. (ed.). 2012. Standard symbolic codes for institutional resource collections in herpetology and ichthyology: An online reference. Version 4.0 (28 June 2013). Electronically accessible at http://www.asih.org/resources. Bird specimens must be deposited at the University of Arizona or other accredited university or museum. Specimens whose collection was intended primarily for a teaching collection at an accredited institution may be housed at the institution where they are used. Information on specimen disposition shall be included in the year-end collecting report.

F) The maximum number of animals that may be collected under the license will apply to the aggregate of all collectors (licensee and agents).

G) Disperse your collection activities for all species to avoid negatively impacting local populations.

H) Survey and capture of wildlife in aquatic habitats must follow the "Field Work Disease Prevention Protocol" in Appendix G of the "Chiricahua Leopard Frog (*Rana chiricahuensis*) Recovery Plan" to prevent spread of disease: http://ecos.fws.gov/docs/recovery_plan/070604_v3.pdf.

I) Do not use destructive collecting techniques (e.g., destroying rock crevices, removing caprock, tearing apart deadfall, etc.). It is unlawful to use manual or powered jacking or prying devices to take reptiles or amphibians (Commission Rule R12-4-303).

J) The license number must be acknowledged in any publications or reports resulting from activities conducted under the authority of this license. A copy of all publications or reports resulting from those activities must be provided to the Department (send to sclicenses@azgfd.gov).

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Appendix E: HACCP plan for San Bernardino/ Leslie Canyon National Wildlife Refuges.

HACCP Step 1 – Activity Description

Activity Description	
Facility: *San Bernardino/Leslie Canyon National Wildlife Refuges*	Site: *All of Arizona*
Project Coordinators: *Bill Radke*	Activity/Management Objective: *Prevent the transfer or exchange of nuisance and invasive aquatic species among statewide waters during fish, frog, and invertebrate population surveys and relocations.*
Site Managers: *Bill Radke*	
Address: *7628 N. Highway 191* Douglas, AZ 85608	
Phone: *520-364-2104*	

HACCP Step 2 – Identify Potential Hazards

(to be transferred to column 2 of HACCP Step 4 – Hazard Analysis Worksheet)

Project Description i.e. Who; What; Where; When; How; Why
Who: *U.S. Fish and Wildlife personnel, contractors and volunteers*
What: *We wish to prevent nuisance and invasive species transfer among statewide waters during fish, amphibian, and invertebrate surveys (includes collection of research specimens)*
Where: *Seasonal population surveys involve sampling of multiple Arizona waters during the year, including (but not limited to) isolated refuge ponds, stock tanks, wetlands, springs/seeps, streams, rivers, ponds, lakes, and reservoirs.*
When: *Surveys are conducted annually with the majority occurring between March and November.*
How: *A combination of sampling methods will be used depending on the target species and site habitat, but may include any of the following:* • *Backpack electrofishing, seine nets of various sizes, dip nets, minnow traps and hoop nets are used for stream/river surveys and small pond/spring/wetland sampling for native fishes.* • *Gill, experimental, or trammel netting for native fish surveys in standing waters and may be set a variety of ways to maximize catch rates.* • *Waders and/or water shoes/boots are worn during most native fish surveys and some invertebrate and amphibian surveys. Measuring boards, weighing scales and trays, dip nets, tape reels, pin flags, PIT tag scanners, buckets, flowmeters, Secchi disks, bait nets, and water quality combo testers are also used.* • *Captured fish may be retained in "livewell" coolers or buckets during sampling. They are then weighed and measured and returned alive to the water at the end of each sampling reach.* • *Aquatic surveys for snails and freshwater mussels involve the use of magnifers, flashlights, forceps, survey rings, artificial substrate tiles, pin flags, tape reels, water quality combo testers, filter buckets, and buckets.*
Why: *Monitoring fish, amphibians, and invertebrate populations in waters in Arizona is critical for obtaining and maintaining data sets in which to base management decisions on conservation and recovery actions, future management needs, and status and trend information for species status reviews.*

Appendix E: continued.

Vertebrates: *Fish, amphibians and turtles in all life stages*
Invertebrates: *Aquatic invertebrates and zooplankton (such as springsnails, native mussels, Asiatic clams, New Zealand mudsnails, apple snails, Chinese mystery snails, ramshorn snails, crayfish and undesirable zooplankton and veligers)*
Plants: *Aquatic macrophytes [such as giant salvinia, Hydrilla, watermilfoils (Eurasian and parrotfeather), pondweeds, naiads, coontail] and phytoplankton (such as golden algae, filamentous and blue-green algae)*
Other Biologics (e.g. genetics, disease, pathogen, parasite, or non-pathogens): *Fish parasites (such as Eurasian tapeworm, leeches and flukes, anchorworms, etc.);* *Aquatic pathogens and diseases (such as Whirling Disease, Largemouth Bass Virus, Bd, etc)*
Other (non-biological contaminants e.g. pesticide residue, oil products, etc. or harborage via packing or construction materials, etc.): *Pesticide residues or undiluted cleaning solutions*

Appendix E: continued.

HACCP Step 3 – Flow Diagram

Flow Diagram Outlining Sequential Tasks to Complete Activity/Project
Described in HACCP Step 1 – Activity Description
(to be transferred to column 1 of the HACCP Step 4 – Hazard Analysis Worksheet)

Task 1	Pre-survey inspection of equipment, waders/boots and vehicles at HQ office
⇓	
Task 2	Travel to water body and conduct the survey
⇓	
Task 3	If applicable, conduct surveys at multiple sites within drainage or watershed where transfer of undesirables may occur or are already present
⇓	
Task 4	Visual inspection of survey equipment, waders/boots and vehicles before leaving the site following the survey. Remove mud, algae, and vegetation fragments – or returning to the office for complete cleaning of gear
⇓	
Task 5	Complete cleaning of equipment, waders/boots and vehicles before deploying on a different body of water * (either field disinfection or HQ wash station use, depending on field work)

* "Different body of water" includes: sites in other drainages or watersheds; isolated waters within the same drainage or watershed sampled; or upstream reaches of a stream or river--separated by a functional fish barrier--that do not have undesirable organisms or contaminants which may occur downstream of the barrier.

Appendix E: continued.

HACCP Step 4 - Hazard Analysis Worksheet

1 Tasks (from HACCP Step 3 - Flow Diagram)	2 Potential hazards identified in HACCP Step 2	3 Are any potential hazards significant? (yes/no)	4 Justify evaluation for column 3	5 What control measures can be applied to prevent undesirable results?	6 Is this task a critical control point? (yes/no)
Task 1 Pre-survey inspection of equipment, waders/boots and vehicles at HQ office	Vertebrates Fish, amphibians and turtles	No	Desiccation, heat and removal of all residual water eliminates likelihood of survival, large organisms are easy to detect and remove		N/A
	Invertebrates Aquatic invertebrates and zooplankton	Yes	Mollusks and zooplankton cysts may survive periods of desiccation	Visually inspect for organisms and ensure equipment is clean and dry	Yes
	Plants Aquatic macrophytes, algae and phytoplankton	Yes	Plant materials, seeds and spores may survive periods of desiccation	Visually inspect for organisms and ensure equipment is clean and dry	Yes
	Other Biologics Fish parasites, aquatic pathogens and diseases	No	Desiccation, heat and removal of all residual water eliminates likelihood of survival		N/A
	Other Pesticide residues or undiluted cleaning solutions	No	Equipment is properly cleaned and stored away from potential contaminants	Clean and rinse gear per BMP6 – effective treatments for AIS	No

Appendix E: continued.

HACCP Step 4 - Hazard Analysis Worksheet (continued)

1 Tasks (from HACCP Step 3 - Flow Diagram)	2 Potential hazards identified in HACCP Step 2	3 Are any potential hazards significant ? (yes/no)	4 Justify evaluation for column 3	5 What control measures can be applied to prevent undesirable results?	6 Is this task a critical control point? (yes/no)
Task 2 Travel to water body and conduct the survey	Vertebrates Aquatic invertebrates and zooplankton	No	Likelihood of re-infection during transit is remote		N/A
	Invertebrates Aquatic invertebrates and zooplankton	No	Likelihood of re-infection during transit is remote		N/A
	Plants Aquatic macrophytes, algae and phytoplankton	No	Likelihood of re-infection during transit is remote		N/A
	Other Biologics Fish parasites, aquatic pathogens and diseases	No	Likelihood of re-infection during transit is remote		N/A
	Other Pesticide residues or undiluted cleaning solutions	No	Likelihood of re-infection during transit is remote		N/A

Appendix E: continued.

HACCP Step 4 - Hazard Analysis Worksheet (continued)

1 Tasks (from HACCP Step 3 - Flow Diagram)	2 Potential hazards identified in HACCP Step 2	3 Are any potential hazards significant ? (yes/no)	4 Justify evaluation for column 3	5 What control measures can be applied to prevent undesirable results?	6 Is this task a critical control point? (yes/no)
Task 3 If applicable, conduct surveys at multiple sites within drainage or watershed where transfer of undesirables may occur or are already present	Vertebrates Fish, amphibians and turtles	No	Survey equipment, waders/boots and vehicles may harbor nuisance species as a result of the survey	Conduct "field" cleaning: visually inspect, remove organic material, use Quat solution to disinfect all items in contact with site water	No
	Invertebrates Aquatic invertebrates and zooplankton	No	Survey equipment, waders/boots and vehicles may harbor nuisance species as a result of the survey	Conduct "field" cleaning: visually inspect, remove organic material, use Quat solution to disinfect all items in contact with site water	No
	Plants Aquatic macrophytes, algae and phytoplankton	No	Survey equipment, waders/boots and vehicles may harbor nuisance species as a result of the survey	Conduct "field" cleaning: visually inspect, remove organic material, use Quat solution to disinfect all items in contact with site water	No
	Other Biologics Fish parasites, aquatic pathogens and diseases	No	Survey equipment, waders/boots and vehicles may harbor nuisance species as a result of the survey	Conduct "field" cleaning: visually inspect, remove organic material, use Quat solution to disinfect all items in contact with site water	No
	Other Pesticide residues or undiluted cleaning solutions	No	Equipment was cleaned and stored properly before current use	Clean and rinse gear per BMP6 – effective treatments for AIS	No

Appendix E: continued.

HACCP Step 4 - Hazard Analysis Worksheet (continued)

1 Tasks (from HACCP Step 3 - Flow Diagram)	2 Potential hazards identified in HACCP Step 2	3 Are any potential hazards significant? (yes/no)	4 Justify evaluation for column 3	5 What control measures can be applied to prevent undesirable results?	6 Is this task a critical control point? (yes/no)
Task 4 Visual inspection before leaving the site following the survey (survey equipment, waders/boots and vehicles). Remove mud, algae, and vegetation fragments Or returning to the office for complete cleaning of gear	Vertebrates Fish, amphibians and turtles	Yes	Survey equipment, waders/boots and vehicles may harbor nuisance species as a result of the survey	Conduct "field" cleaning: visually inspect, remove organic material, use Quat solution to disinfect all items in contact with site water	No
	Invertebrates Aquatic invertebrates and zooplankton	Yes	Survey equipment, waders/boots and vehicles may harbor nuisance species as a result of the survey	Conduct "field" cleaning: visually inspect, remove organic material, use Quat solution to disinfect all items in contact with site water	No
	Plants Aquatic macrophytes, algae and phytoplankton	Yes	Survey equipment, waders/boots and vehicles may harbor nuisance species as a result of the survey	Conduct "field" cleaning: visually inspect, remove organic material, use Quat solution to disinfect all items in contact with site water	No
	Other Biologics Fish parasites, aquatic pathogens and diseases	Yes	Survey equipment, waders/boots and vehicles may harbor nuisance species as a result of the survey	Conduct "field" cleaning: visually inspect, remove organic material, use Quat solution to disinfect all items in contact with site water	No
	Other Pesticide residues or undiluted cleaning solutions	No	Equipment was cleaned and stored properly before current use	Clean and rinse gear per BMP6 – effective treatments for AIS	No

Appendix E: continued.

HACCP Step 4 - Hazard Analysis Worksheet (continued)

1 Tasks (from HACCP Step 3 - Flow Diagram)	2 Potential hazards identified in HACCP Step 2	3 Are any potential hazards significant? (yes/no)	4 Justify evaluation for column 3	5 What control measures can be applied to prevent undesirable results?	6 Is this task a critical control point? (yes/no)
Task 5 Complete cleaning of equipment, waders/boots and vehicles before deploying on a different body of water (either field disinfection or HQ wash station use, depending on field work)	Vertebrates Fish, amphibians and turtles	No	Desiccation, heat and removal of all residual water eliminates likelihood of survival; large organisms are easy to detect and remove		N/A
	Invertebrates Aquatic invertebrates and zooplankton	Yes	Mollusks and zooplankton cysts may survive periods of desiccation	Conduct "office" cleaning: use Quat solution to disinfect all items in contact with site water, completely dry and visually inspect gear before storing.	Yes
	Plants Aquatic macrophytes, algae and phytoplankton	Yes	Plant materials, seeds and spores may survive periods of desiccation	Conduct "office" cleaning: use Quat solution to disinfect all items in contact with site water, completely dry and visually inspect gear before storing.	Yes
	Others Biologics Fish parasites, aquatic pathogens and diseases	No	Desiccation, heat and removal of all standing water eliminates likelihood of survival	Clean and rinse gear per BMP6 – effective treatments for AIS	N/A
	Others Pesticide residues or undiluted cleaning solutions	No	Equipment is well maintained and cleaned	Clean and rinse gear per BMP6 – effective treatments for AIS	N/A

.

Appendix E: continued.

HACCP Plan Form (all CCP's or "yes's" from column 6 of HACCP Step 4 – Hazard Analysis Worksheet)		
(1) Critical Control Point:		Task 1: Pre-survey inspection of equipment, waders/boots and vehicles at HQ office
Significant Hazard(s):		Aquatic invertebrates and zooplankton; aquatic macrophytes, algae and phytoplankton
Limits for Each Control Measure:		Size, visibility, detection of nuisance organisms. Ability to effectively disinfect and completely dry equipment (length of time between surveys)
Monitoring	What:	Survey equipment, waders/boots and vehicles
	How:	Visual inspection and ensure gear is clean and dry before use
	Frequency:	Prior to each survey event
	Who:	Nongame Native Fish and Invertebrate Program staff, contractors and volunteers
Evaluation & Corrective Action(s) (if needed):		Complete additional thorough cleaning as per Task 5 before using gear for survey
Supporting Documents (if any):		HACCP plan and AZGFD's BMP#6 (Appendix A of this document)
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(2) Critical Control Point:		Task 5: Complete cleaning of equipment, waders/boots and vehicles before deploying on a different body of water* (either field disinfection or HQ wash station use, depending on field work)
Significant Hazard(s):		Aquatic invertebrates and zooplankton; aquatic macrophytes, algae and phytoplankton
Limits for Each Control Measure:		Size, visibility, detectability of nuisance organisms. Ability to effectively disinfect and completely dry equipment (length of time between surveys)
Monitoring	What:	Survey equipment, waders/boots and vehicles
	How:	Use Quat solution to disinfect all items in contact with site water, completely dry and visually inspect gear before storing
	Frequency:	Prior to moving to different body of water
	Who:	Nongame Native Fish and Invertebrate Program staff, contractors and volunteers
Evaluation & Corrective Action(s) (if needed):		Complete thorough second cleaning as described above
Supporting Documents (if any):		HACCP plan and AZGFD's BMP#6 (Appendix A of this document)
>>><<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<		

Facility: Arizona Game and Fish Dept Headquarters	**Activity/Management Objective:** HACCP Plan was followed; date and disinfected gear noted on HACCP cleaning record in the warehouse.
Address: 5000 W Carefree Hwy, WMNG, Phoenix, AZ 85086	
Signature:	**Date:**

Appendix E: continued.

Appendix A

Effective treatments for aquatic invasive species found in the Intermountain West and Southwest. Copied from Table 1 of the "Technical Guidelines for AIS Prevention 11-08.doc"

Aquatic Invasive Species	Wash and remove organics (e.g. mud)	Temperature	Drying	Bleach (e.g. Clorox®) 6% sodium hypochlorite (NaClO)	Quaternary ammonium compounds [e.g. n-alkyl dimethyl benzyl ammonium chloride (ADBAC)
Whirling Disease	Yes	90 °C (195 °F); 10 min	Be dry for 24 h, in sunlight best	For 10 min: 1% bleach solution (1 oz/ 1gal water)	For 10-15 minutes: Quat 128 (6oz/1gal)
Viral Hemorrhagic Septicemia (VHS), other viruses	Thoroughly wash	46°C (120°F); 5 min Inactive after 24 hours at 20°C (68° F)	Be dry for 24 h, in sunlight best	For 10 min soak or circulate: 1% bleach solution (1 oz/1gal water)	Unknown, but likely effective. For 10-15 minutes soak or circulate: Quat 128 (6oz/1gal)
Amphibian Chytrid Fungus	Yes	60°C (140°F); 5 min	Be dry for 3 hr, in sunlight best	For 30 sec: 20% solution (22oz/1 gal) -or- for 10 min: 7% solution 9oz/1gal	For 30 sec: Quat 128 (1/8 tsp/1gal)
New Zealand Mudsnails	Yes	46°C (120°F); 5 min	Be dry for 48 hr, in sunlight best	Not effective	For 10-15 minutes: Quat 128 (6oz/1gal)
Zebra/Quagga Mussels	Yes, pressure wash flushes veligers	≥140°F water	3-30 days, in sunlight best	For 1 min: 0.5% bleach solution (1/2 oz/1gal water)	No data, but likely effective
Didymo (aka: "rock snot")	Yes	60°C (140°F); 1 min	Be dry for 48 h, in sunlight best	For 1 min: 2% bleach solution (2 oz/ 1gal water)	No data, but likely effective
Golden Alga	Thoroughly wash	>104°F	Be dry for 2-3 days in direct sunlight	For 24 h at 62.5-500 mg/l (0.01-0.07 oz/gal); 1 h at 3,125 mg/l (0.42 oz/gal); or 15 min at 12,500 mg/l (1.67 oz/gal).	No data, but likely effective
Giant Salvinia	Yes	>43°C (109°F) or < -3°C (26 °F) for > 2 hrs	Uncertain, but dry at least 48 h, in sunlight best	No data, but likely effective.	No data, but likely effective
Eurasian Watermilfoil and Parrot Feather	No data but likely killed with>60°C (140°F)	Uncertain, but completely dry at least 48 h, in sunlight best	No data, but likely effective.	No data, but likely effective	No data but likely effective

Appendix E: continued.

Aquatic Invasive Species	Wash and remove organics (e.g. mud)	Temperature	Drying	Bleach (e.g. Clorox®) 6% sodium hypochlorite (NaClO)	Quaternary ammonium compounds [e.g. n-alkyl dimethyl benzyl ammonium chloride (ADBAC)
Hydrilla	Yes	No data but likely killed with>60°C (140°F)	Uncertain, but dry at least 48 h, in sunlight best	No data, but likely effective.	No data, but likely effective
Fish & Amphibians	Yes	≥140°F water	Be dry for 3 hr, in sunlight best	For 30 sec: 20% solution (22oz/1 gal)	Acute toxicity (EPA)
Crayfish	Yes	≥140°F water	Be dry for 3 hr, in sunlight best	For 30 sec: 20% solution (22oz/1 gal)	No data, but likely effective as ADBAC is toxic to most aquatic organisms
Other	(Similar species of snails, plants, pathogens, and vertebrate and invertebrate invasive species) No data but treatments for whirling disease and/or New Zealand mudsnails are likely effective				

(AZGFD note: if Quat128 or Formula 409 cleaning solutions are not available, use full strength distilled white vinegar. Bleach solutions are less preferred for cleaning boats and some equipment because they can be very corrosive to fabrics, plastics, rubber, and metal.)

Quaternary ammonium compounds, or 'quats', are common disinfectants with an array of uses, from killing algae in swimming pools to sanitizing workout equipment at the gym. They are relatively nontoxic and do not damage fabric, metals, or gaskets. Solutions of quat compounds retain their effectiveness over days and can be reused if not excessively diluted. Disinfection with quaternary ammonium compounds is the recommended treatment for most aquatic invasive species found in the Southwest. These products are labeled for use as fungicides/virucides.

<u>Recipe for 5% cleaning solution using Quat128</u>

One gallon of Quat128 will create 20 1-gallon 5% cleaning solutions

Volume of tap water	Volume of Quat128
1 gallon water	6.35 liquid oz.
1 gallon water	12.7 tbsp
1 gallon water	0.79 cups

Appendix F: R scripts from R package "Depletion".

```
#' Hierarchical multiseason Bayesian Depletion Model
#'
#' Transforms count and habitat data and returns abundance estimates produced
by the Bayesian mixture model
#' @param count Species-specific count
#' @param species Identifies the species being modeled (Yaqui topminnow =
"YTop", Yaqui chub = "YChub", or Mexican longfin dace = "MDace")
#' @return The Bayesian mixture model returns true abundance estimates for
each Stream reach and Year of data collection
#' @export
deplete<-function(count,hab,species=c("YChub,YTop,MDace")){
#Error bounds
if (length(species)>1|missing(species)) stop("'species'must contain only one
value",call.=FALSE)
if (missing(count)) stop("must specify count data",call.=FALSE)
if (missing(hab)) stop("must specify habitat data",call.=FALSE)

#Reorganze count data by Year and Stream reach
#Capture count data by species
if(species=="YChub"){
   sort.dat<-count[
     with(count,order(count$Year,count$Site))
  ]

countY=cbind(sort.dat$GIPU_1,sort.dat$GIPU_2,sort.dat$GIPU_3,sort.dat$GIPU_4,
sort.dat$GIPU_5,sort.dat$GIPU_6,

sort.dat$GIPU_7,sort.dat$GIPU_8,sort.dat$GIPU_9,sort.dat$GIPU_10)
 }else if(species=="YTop"){
   sort.dat<-count[
     with(count,order(count$Year,count$Site))
  ]

countY=cbind(sort.dat$POSU_1,sort.dat$POSU_2,sort.dat$POSU_3,sort.dat$POSU_4,
sort.dat$POSU_5,sort.dat$POSU_6,

sort.dat$POSU_7,sort.dat$POSU_8,sort.dat$POSU_9,sort.dat$POSU_10)
 }else if(species=="MDace"){
   sort.dat<-count[
     with(count,order(count$Year,count$Site))
  ]

countY=cbind(sort.dat$AGCH_1,sort.dat$AGCH_2,sort.dat$AGCH_3,sort.dat$AGCH_4,
sort.dat$AGCH_5,sort.dat$AGCH_6,

sort.dat$AGCH_7,sort.dat$AGCH_8,sort.dat$AGCH_9,sort.dat$AGCH_10)
  }

#Define array dimensions
#nsite = the total number of Stream reaches sampled
#nrep = the total number of depletion passes for each Stream reach and Year
#nyear = the number of years that each Stream reach was surveyed
nsite=length(as.factor(unique(sort.dat$Site)))
nrep=ncol(countY)
nyear=length(as.factor(unique(sort.dat$yr)))

#Create empty three-dimensional array
#"NA" is used as a placeholder in the array. Below, we will replace "NA" with
the observed data.
dataCount = array(NA,dim=c(nsite,nrep,nyear))

#Read in countY data into three-dimensional array.
for(i in 1:nyear){
```

```
    dataCount[,,i]=countY[((i-1)*nsite+1):(i*nsite),]
 }


#Reorganize habitat data by Year and Site
sort.hab<-hab[
    with(hab,order(hab$Year,hab$Site)),
 ]

#Capture the set of measurements and then calculate mean (rowMeans)
#for each environmental variable for each Site and Year

#Channel Unit

CHUnit=cbind(sort.hab$CHUnit_0,sort.hab$CHUnit_5,sort.hab$CHUnit_10,sort.hab$
CHUnit_15,sort.hab$CHUnit_20,sort.hab$CHUnit_25)

CHUnit=rowMeans(CHUnit)

#Create two-dimensional array
CHUnit=matrix(CHUnit,dim=c(nsite,nyear))

#Channel width

CHWidth=cbind(sort.hab$CHWidth_0,sort.hab$CHWidth_5,sort.hab$CHWidth_10,sort.
hab$CHWidth_15,sort.hab$CHWidth_20,sort.hab$CHWidth_25)

CHWidth=rowMeans(CHWidth)
CHWidth=matrix(CHWidth,dim=c(nsite,nyear))

#Water temperature

WTemp=cbind(sort.hab$WTemp_0,sort.hab$WTemp_5,sort.hab$WTemp_10,sort.hab$WTem
p_15,sort.hab$WTemp_20,sort.hab$WTemp_25)

WTemp=rowMeans(WTemp)
WTemp=matrix(WTemp,dim=c(nsite,nyear))

#Water turbidity

Wturbidity=cbind(sort.hab$WTurb_0,sort.hab$WTurb_5,sort.hab$WTurb_10,sort.hab
$WTurb_15,sort.hab$WTurb_20,sort.hab$WTurb_25)

WTurbidity=rowMeans(WTurbidity)
WTurbidity=matrix(WTurbidity,dim=c(nsite,nyear))

#Water algal

Walgal=cbind(sort.hab$Walgal_0,sort.hab$Walgal_5,sort.hab$Walgal_10,sort.hab$
Walgal_15,sort.hab$Walgal_20,sort.hab$Walgal_25)

Walgal=rowMeans(Walgal)
Walgal=matrix(Walgal,dim=c(nsite,nyear))

#Water conductivity

WCond=cbind(sort.hab$WCond_0,sort.hab$WCond_5,sort.hab$WCond_10,sort.hab$WCon
d_15,sort.hab$WCond_20,sort.hab$WCond_25)

WCond=rowMeans(WCond)
WCond=matrix(WCond,dim=c(nsite,nyear))

#Fine substrate
```

```
SubFine=cbind(sort.hab$SubFine_0,sort.hab$SubFine_5,sort.hab$SubFine_10,sort.
hab$SubFine_15,sort.hab$SubFine_20,sort.hab$SubFine_25)

SubFine=rowMeans(SubFine)
SubFine=matrix(SubFine,dim=c(nsite,nyear))


#Gravel substrate

SubGravel=cbind(sort.hab$SubGravel_0,sort.hab$SubGravel_5,sort.hab$SubGravel_
10,sort.hab$SubGravel_15,sort.hab$SubGravel_20,sort.hab$SubGravel_25)

SubGravel=rowMeans(SubGravel)
SubGravel=matrix(SubGravel,dim=c(nsite,nyear))

#Cobble substrate

SubCobble=cbind(sort.hab$SubCobble_0,sort.hab$SubCobble_5,sort.hab$SubCobble_
10,sort.hab$SubCobble_15,sort.hab$SubCobble_20,sort.hab$SubCobble_25)

SubCobble=rowMeans(SubCobble)
SubCobble=matrix(SubCobble,dim=c(nsite,nyear))

#Boulder substrate

SubBoulder=cbind(sort.hab$SubBoulder_0,sort.hab$SubBoulder_5,sort.hab$SubBoul
der_10,sort.hab$SubBoulder_15,sort.hab$SubBoulder_20,sort.hab$SubBoulder_25)

SubBoulder=rowMeans(SubBoulder)
SubBoulder=matrix(SubBoulder,dim=c(nsite,nyear))

#Stream velocity

StVelocity=cbind(sort.hab$StVelocity_0_1,sort.hab$StVelocity_0_2,sort.hab$StV
elocity_0_3,sort.hab$StVelocity_0_4,sort.hab$StVelocity_5_1,sort.hab$StVeloci
ty_5_2,sort.hab$StVelocity_5_3,sort.hab$StVelocity_5_4,sort.hab$StVelocity_10
_1,sort.hab$StVelocity_10_2,sort.hab$StVelocity_10_3,sort.hab$StVelocity_10_4
,sort.hab$StVelocity_15_1,sort.hab$StVelocity_15_2,sort.hab$StVelocity_15_3,s
ort.hab$StVelocity_15_4,sort.hab$StVelocity_20_1,sort.hab$StVelocity_20_2,sor
t.hab$StVelocity_20_3,sort.hab$StVelocity_20_4,sort.hab$StVelocity_25_1,sort.
hab$StVelocity_25_2,sort.hab$StVelocity_25_3,sort.hab$StVelocity_25_4)

StVelocity=rowMeans(StVelocity)
StVelocity=matrix(StVelocity,dim=c(nsite,nyear))

#Water depth

WDepth=cbind(sort.hab$WDepth_0_1,sort.hab$WDepth_0_2,sort.hab$WDepth_0_3,sort
.hab$WDepth_0_4,sort.hab$WDepth_5_1,sort.hab$WDepth_5_2,sort.hab$WDepth_5_3,s
ort.hab$WDepth_5_4,sort.hab$WDepth_10_1,sort.hab$WDepth_10_2,sort.hab$WDepth_
10_3,sort.hab$WDepth_10_4,sort.hab$WDepth_15_1,sort.hab$WDepth_15_2,sort.hab$
WDepth_15_3,sort.hab$WDepth_15_4,sort.hab$WDepth_20_1,sort.hab$WDepth_20_2,so
rt.hab$WDepth_20_3,sort.hab$WDepth_20_4,sort.hab$WDepth_25_1,sort.hab$WDepth_
25_2,sort.hab$WDepth_25_3,sort.hab$WDepth_25_4)

WDepth=rowMeans(WDepth)
WDepth=matrix(WDepth,dim=c(nsite,nyear))


modelFilename="david_stewart_sanBstream_bayes.txt"
 cat('
     model{
```

```
#Priors for abundance model
beta~dnorm(0,0.01) #Prior for intercept
beta.SubGravel~dnorm(0,0.01) #Prior for slope of Substrate Gravel
beta.SubFine~dnorm(0,0.01) #Prior for slope of Substrate Fine
beta.WDepth~dnorm(0,0.01) #Prior for slope of Water Depth
beta.CHWidth~dnorm(0,0.01) #Prior for slope of Channel Width
beta.CHUnit~dnorm(0,0.01) #Prior for slope of Channel Unit
beta.StVelocity~dnorm(0,0.01) #Prior for slope of Stream Velocity
phi~dunif(0.01,100) #Prior for overdispersion parameter (site-specific
variation)

#Priors for detection model
for(i in 1:nsite){
  for(k in 1:nyear){
    q0[i,k]~dunif(0,1)
    a[i,k]~dunif(0,1)
    }
  }
alpha~dnorm(0,0.01) #Prior for intercept
alpha.SubGravel~dnorm(0,0.01) #Prior for slope of Substrate Gravel
alpha.CHUnit~dnorm(0,0.01) #Prior for slope of Channel Unit
alpha.StVelocity~dnorm(0,0.01) #Prior for slope of Stream Velocity
alpha.CHWidth~dnorm(0,0.01) #Prior for slope of Channel Width

#Priors for temporal random effect
gamma~dnorm(0,0.001)

#Likelihood
for(i in 1:nsite){
eta[i]~dgamma(phi,phi) #Prior for Gamma latent variable
for(k in 1:nyear){

#Ecological model for true abundance
N[i,1,k]<-N.total[i,k]
N.total[i,k]~dpois(lambda[i,k])
lambda[i,k]<-mu[i,k]*eta[i]
log(mu[i,k])<-beta + beta.SubGravel*SubGravel[i,k] +
beta.SubFine*SubFine[i,k] + beta.WDepth*WDepth[i,k] +
beta.CHWidth*CHWidth[i,k] + beta.CHUnit*CHUnit[i,k] +
beta.StVelocity*StVelocity[i,k] + theta[i,k]

for(j in 1:nrep){
#Observation model for removal count data
counts.multi[i,j,k]~dbin(q[i,j,k],N[i,j,k])
N[i,j+1,k]<-N[i,j,k]-counts.multi[i,j,k]
}
}
}

#Detection model
for(i in 1:nsite){
for(k in 1:nyear){
for(j in 1:nrep){
q[i,j,k]<-q1[i,k]+(q0[i,k]-q1[i,k])*(1-pow(a[i,k],(j-1)))
#q[i,j,k]<-q1[i,k]*((1-q0[i,k])^(j-1))
#q[i,j,k]<-q1[i,k]*((1-q1[i,k])^(j-1))
}
}
logit(q1[i,k])<-alpha + alpha.SubGravel*SubGravel[i,k] +
alpha.CHUnit*CHUnit[i,k] + alpha.StVelocity*StVelocity[i,k] +
alpha.CHWidth*CHWidth[i,k]
}

#Temporal random effects
```

```
      for(i in 1:nsite){
      theta[i,1]<-N.total[i,1]
      for(k in 2:nyear){
      theta[i,k]<-mu[i,k]+gamma*(totalC[i,k-1]-mu[i,k-1])
      }
      }
}',fill=TRUE,file=modelFilename)

#Initial abundance values
Nst=apply(dataCount,c(1,3),sum,na.rm=TRUE)+1
jags.inits=function(){
    list(N.total=Nst,alpha=rnorm(1,0,1),beta=rnorm(1,0,1))
  }

#Bundle data

jags.data=list(counts.multi=dataCount,totalC=Nst,nsite=dim(dataCount)[1],nrep
=dim(dataCount)[2],nyear=dim(dataCount)[3])

#Parameters monitored
jags.params=c("N.total","gamma","q1")

#MCMC settings
ni=500000;nb=20000;nt=2;nc=4

#Call JAGS

jagsfit=autojags(data,inits,parameters.to.save=params,model.file=modelFilenam
e,n.chains=nc,n.adapt=1000,iter.increment=10000,n.burnin=nb,n.thin=nt,save.al
l.iter=FALSE,factories=NULL,parallel = TRUE,n.cores=8,DIC=TRUE,
Rhat.limit=1.1,max.iter=ni,verbose=TRUE)

#Create Year labels
yrlab<-seq(min(sort.dat$Year),max(sort.dat$Year),by=1)
yrlab<-rep(yrlab,nsite)

#Create Site labels
site.name<-rep(as.character(unique(unlist(sort.dat$Site))),nyear)

#Summarize posteriors for abundance
N.total<-round(unlist(jagsfit$mean$N.total))
N.total<-as.vector(N.total)
N.lower<-unlist(jagsfit$q2.5$N.total)
N.lower<-as.vector(N.lower)
N.upper<-unlist(jagsfit$q97.5$N.total)
N.upper<-as.vector(N.upper)

#Use data.frame to package results to save to working directory
res<-
data.frame(Site=site.name,Year=yrlab,Lower95=N.lower,Pop_estimate=N.total,Upp
er95=N.upper)

res<-res[
    with(res,order(res$Site,res$Year)),
  ]


if(species=="YChub"){

#Capture and Write results to working directory
write.csv(res,"YaquiChubStreamAbundance.csv",row.names=F)

plot<-ggplot(res,aes(Year,Pop_estimate,colour=factor(Site)))+
   geom_point(size=4)+
```

```
   geom_errorbar(aes(ymin=Lower95,ymax=Upper95),width=.3)+
   facet_wrap(~Site,ncol=2)+
   guides(colour="none")+
   theme_bw()+
   xlab("Year")+
   ylab("Abundance")+
   theme(axis.text=element_text(size=12),
         axis.title = element_text(size=16),
         strip.text.x=element_text(size=12))
print(plot)
ggsave("YaquiChubStreamAbundanceFigure.tiff",plot=plot,dpi=300)
return(res)

}else if(species=="YTop"){

#Capture and Write results to working directory
write.csv(res,"YaquiTopminnowStreamAbundance.csv",row.names=F)

plot<-ggplot(res,aes(Year,Pop_estimate,colour=factor(Site)))+
   geom_point(size=4)+
   geom_errorbar(aes(ymin=Lower95,ymax=Upper95),width=.3)+
   facet_wrap(~Site,ncol=2)+
   guides(colour="none")+
   theme_bw()+
   xlab("Year")+
   ylab("Abundance")+
   theme(axis.text=element_text(size=12),
         axis.title = element_text(size=16),
         strip.text.x=element_text(size=12))
print(plot)
ggsave("YaquiTopminnowStreamAbundanceFigure.tiff",plot=plot,dpi=300)
return(res)

}else if(species=="MDace"){

#Capture and Write results to working directory
write.csv(res,"MexicanLDaceStreamAbundance.csv",row.names=F)

plot<-ggplot(res,aes(Year,Pop_estimate,colour=factor(Site)))+
   geom_point(size=4)+
   geom_errorbar(aes(ymin=Lower95,ymax=Upper95),width=.3)+
   facet_wrap(~Site,ncol=2)+
   guides(colour="none")+
   theme_bw()+
   xlab("Year")+
   ylab("Abundance")+
   theme(axis.text=element_text(size=12),
         axis.title = element_text(size=16),
         strip.text.x=element_text(size=12))
print(plot)
ggsave("MexicanLDaceStreamAbundanceFigure.tiff",plot=plot,dpi=300)
return(res)
  }
}
```

Appendix G: Record of I&M Protocol Peer-Review

Protocol Title: Survey Protocol Framework for Monitoring Abundance of Río Yaqui Fishes in Streams Version 1.1
Survey Identification Number:
Refuge: San Bernardino and Leslie Canyon National Wildlife Refuges
Authors: David R. Stewart, Statistician, U.S. Fish and Wildlife Service
Lacrecia A. Johnson, Zone Biologist, U.S. Fish and Wildlife Service
Cinthia Eichhorn, Regional Data Manager, U.S. Fish and Wildlife Service

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Protocol Review Timeline

10/10/2018 – Protocol submitted for peer review.

10/25/2018 – Peer review (1 external to USFWS (Don Mitchell, Aquatic Program Supervisor, Arizona Game and Fish Department) received and returned to authors.

11/13/2018 – Protocol revision received from authors.

Letter to Dr. Metzger:

11/13/2018

Dear Dr. Metzger,

We have completed revision of the Inventory and Monitoring protocol entitled "Survey Protocol Framework for Monitoring Abundance of Río Yaqui Fishes in Streams". Below we have attached the reviews and our response to questions, edits, or suggestions raised by the Reviewer. All comments from the Reviewer have been addressed in this document (see blue text) or within the protocol.

We believe this critique has helped us develop a better protocol. We appreciate and thank the Reviewer for their time and effort that they invested to help further this document.

Sincerely,

David R. Stewart

Letter From Dr. Metzger (with authors' responses in blue):

10/25/2018

Hi Kris, Please find attached my comments/suggestions related to the Rio Yaqui Stream Survey Protocol. The document is in Excel format currently however if you require a different format let me know.

If you need anything further let me know.

Thanks, for the opportunity to provide input, I look forward to seeing the final product!

We have incorporated almost all of the comments and suggestions provided by the Reviewer. We feel that these comments greatly improved the protocol, and greatly appreciate the time and consideration that they invested to help further this protocol to completion.

Don Mitchell
Aquatic Program Supervisor, RV
Arizona Game and Fish Department
555 N. Greasewood Rd.
Tucson, AZ. 85743
Ofc: (520) 388-4451
Fax: (520) 628-5080
dmitchell@azgfd.gov

Specific comments

1. P7, L16 – change grater to greater

   We changed grater to greater.

2. P13, L19-21 – I realize that this is a stream protocol however, the vast majority of the fish population on the El Coronado reside in the numerous ponds located on the ranch. How will those populations be monitored in the future?

   We are developing and finalizing a formal survey protocol to estimate the true abundance of Río Yaqui fishes in ponds.

3. P13, L41 – The process of site selection is confusing. Will the entire reach be surveyed in 25m increments or will a sub sample of the reach be sampled? The reaches are known but how will the sites within the reaches be determined?

   The reach by our definition is the study site and measures 25 m in length. The "streams" are known and the stream "reaches" are pre-determined based on >10 years of historical survey data. We edited the sentences for clarity.

4. P13-14, L44 – These sentences contradict each other. One says they have not expanded and the following sentences says analysis will depend on expansion. How will expansion be detected? I would suggest random sites outside of known occupied habitat be included to detect expansion.

   We agree with the reviewer that the sentences are confusing. We edited this section for clarity. First, the predetermined sites include sites that are located immediately downstream of these that have at least one of the four Río Yaqui fish species. These sites were selected because to detect downstream expansion. They are located 50 to 100 m downstream. These sites have been sampled for >10 years with zero detection of expansion. We have added this to the protocol.

5. P14, L27 – change maintain to maintaining

   We incorporated this change.

6. P16, L6 – AZGFD prefers to have a minimum of 3 crewmembers, 2 with CPR/ first aid with one of the trained crewmembers not actively involved with the electroshocking.

   We agree with the reviewer. However, and because of staff limitations, we occasionally have no more than 2 crewmembers during a survey. Therefore we are forced to modify the survey to also apply to those years when the Refuge might be staff limited. The sentence now reads, "At-least two crewmembers on an electroshocking team must have a current certificate in CPR and First Aid Training. If possible, and in the event of three crewmembers, one of the two trained crewmembers must be stationed on the bank during the survey."

7. P17, L3 – This section is confusing. If the sites are known and pre-determined then provide maps with locations.

   We did not incorporate a map with the locations. This protocol applies to those predetermined stream reaches found at San Bernardino National Wildlife Refuge and also to those located on private land, and we decided against identifying the specific locations of these stream reaches out of respect to the private land owners and also to protect the location of these sensitive species because this protocol will be freely available to the general public.

8. P17, L8 – All stream pre-determined stream reaches will be surveyed.

   We added, "at each predetermined stream reach to the sentence."

9. P17, L9 – Stream surveys should always be conducted in an upstream manner to prevent poor visibility conditions.

   This specific sentence does not speak to how one should approach sampling a specific stream reach (upstream versus downstream). The motivation behind this sentence is that the predetermined stream reaches do not have to be sampled in a specific order. We added information for clarity.

10. P17, L7 – Again because I don't fully understand site selection, will there be any random sites sampled during these surveys?

    The predetermined sampling units (i.e., stream reaches) are intended to remain static across surveys and years. These stream reaches include those known to have Río Yaqui fish and also those located immediately downstream from these areas. Stewart et al. (2019) sampled these and also random stream reaches in and around the area. This study identified that these species only persist in a small number of “known” stream reaches located in SW Arizona.

11. P17, L31 – A better understanding of site selection would help me here.

    The stream reaches are predetermined.

12. P17, L33 – This mesh size is too large for topminnows and even smaller chub. It is recommended that mesh size be no larger than 3mm or 1/8" when setting block nets for these small bodied species.

    The reviewer is correct. Though we already identified that the mesh size should be < 7 mm based on recommendations in Peterson et al. 2004 and other studies, we revisited this sentence and it specifies specifically that the mesh size should be no larger than 3 mm.

13. P17, L35 – Recommend changing …..before sampling…. to ….before <u>each</u> sampling pass.

    We incorporated this change.

14. P17, L43 – Recommend standardizing everything with the downstream end of sampling unit as starting point, where water quality is taken, GPS documentation, etc.

    We incorporated this change.

15. P17, L43 – This is the first mention of "Station ID." Need to better define the entire section on sampling sites, unit reaches.

    We agree with the reviewer. We incorporated this change throughout the document.

16. P18, L6 – Water quality should be taken at the downstream end of the sample site. This should be done first before any entry to the stream. If not possible without entry then it should be downstream of where the lower block net will be established.

    We incorporated this change.

17. P18, L9 – Water quality parameters are not consistent through document. See page 15 line 21.

    We fixed this error so that the same water quality parameters are identified in each section.

18. P18, L31 – Recommend that you mention that buckets should be filled with creek water from a location below sampling station prior to beginning sampling so they are ready and waiting for captured fish.

    We incorporated this change.

19. P18, L34 – The netter(s) is going to be very busy trying to catch fish, pay attention to where the electro unit is, keeping their footing, etc. and the stopwatch is going to add to this. If this is actually needed I recommend the non netter on the shore should be responsible for the stopwatch.

    This is very much needed. It is difficult to keep track of time when the number of "on-time" seconds is located on the back of the electroshocking unit. This is important because some passes may receive greater effort than others depending on the environment, species, and the numbers of fish encountered. For example, when a lot of fish are encountered, it is easier for crewmembers to methodically collect fish and move upstream. This can take considerably more time in comparison to those passes where few fish are captured, or when one implements the final pass of the survey during a long day. Therefore, and to help standardize effort among passes, it is also beneficial for either a crewmember located on shore (as you stated) or the netter equipped with a timing device on the inside of their wrist and in view as another method to keep pace. In doing so, one should have a relative idea of about how long it may take to complete the 300 "on-time" seconds for each pass. This is being implemented to control for survey effort, ensure consistent effort is being applied across all passes, and will help mitigate some of the error associated with heterogeneity in detection probability that we now know can bias the model-based information being produced from depletion assessments (Stewart et al. 2019). We added this to the document.

20. P18, L36 – The 300 seconds, was that actual on-time on the e-unit or time it took to work through the sampling unit. AZGFD standardizes the electroshocking "on-time" in seconds not the time it takes to move through the sampling station. I'm not clear on why the stopwatch is needed.

    The 300 seconds is the actual "on-time" seconds recorded on the electroshocking unit after each pass. The stopwatch will tell us about how long 300 "on-time" seconds takes and is only intended to keep folks aware of how long they are taking to survey the stream reach.

21. P19, L22-29 – This is really confusing. Recommend a flowchart that will allow a better visualization of the process.

    We did not provide a flowchart because this paragraph is only intended to provide a general description of the survey logistics. The step-by-step approach to implement this survey can be found in SOP1.

22. P19, L28 – How will problems with block nets be handled? (e.g. net is blown out during 3rd pass of sampling or is found to not be effective after 5 passes?). Will surveys be halted, repairs made and start over or continue at pint where problem found?

    We added the following to the protocol: "Next, both block nets should be visually inspected after each pass to ensure that nets remain stretched from bank-to-bank and stretched from 12 inches above the water surface to substrate. If at any time the block nets are washed downstream, then sampling should be discontinued for the day, fish should be returned to the stream reach, and the stream reach should be surveyed exactly one week later. If after the second or third pass of sampling the integrity of the block nets change and are no longer stretching from bank to bank, then the surveyors should discontinue sampling, repair the nets, and then continue sampling the stream reach."

23. P28, L10 – Recommend adding need for Scientific collecting permit and adherence to the stipulations and reporting requirements for fish work in the state associated with the permit. One of the stipulations is the requirement for a HACCP plan to prevent spread of aquatic invasives and disease. A HACCP plan should be including as an appendix in this protocol. Bill Radke is familiar with this permitting requirement.

    We added a section to the protocol that specifies that the Lead Biologist is required to attain both a federal and state scientific collection permit. We also identified that the HACCP planning document should be reviewed at the beginning of each season and before each sampling occasion to limit transport of aquatic nuisance species. We attached the 2017 Example State Permit and the HACCP plan as an Appendix to the protocol.

24. P31, L19 – Swift water and high turbidity can have negative impacts on netting efficiency. Consider at what flows and turbidity surveys should not be performed.

    We identified in the Pre-survey logistics and training section that backpack electroshocking should not be attempted if the average depth of water is too deep for operators to wade at less than "thigh depth" for the majority of the exercise (e.g., Black Draw at Leslie Canyon National Wildlife Refuge). Suspend wading operations if adverse weather or water conditions are a safety concern (i.e., thunder, lightning, swift water/ extreme flow conditions). Lastly, do not enter the water if you are unable to swim or are uncomfortable with your swimming abilities. Moreover, swift water/ extreme flow conditions are typically accompanied by excessive run off and associated turbidity in this region, and thus these conditions are accounted for because the Refuge would not survey for safety reasons.

25. P32, L5 – Should the crew establish the survey section in an upstream or downstream direction? Care should be taken to not disturb the sampling site during this step. Crew should work along the edge trying to stay out of the water.

    This is a great point. We added that, "At each stream reach, crews will **establish** a 25 meter sampling unit from the center of the sampling location by working along the bank edge and staying out of the water."

26. P32, L9 – Mesh size for block nets and dip nets should be no larger than 3mm or 1/8"

    We incorporated this change and the mesh size for both block nets and dip nets identifies that the mesh should be no larger than 3 mm or 1/8".

27. P32, L11 – Recommend mention of watching for undercut banks here when setting up block nets.

    We added a Note to SOP1 that identifies a defined set of steps that one should follow in the event that a block net may be stretched across a site having an undercut bank. The idea is that those surveying at the time of the survey would increase the length of the stream reach by 5 m or from 25 m to 30 m, and will continue doing this until the net can be stretched across the stream and will be secured from the presence of an undercut bank that will allow fish to escape the defined population.

28. P32, L12 – Block nets should be a minimum of 12 inches above water surface.

    We added that block nets should be a minimum of 12 inches above water surface throughout the protocol.

29. P32, L30 – Contradicts 18-5. Downstream is the preferred location and prior to any entry.

    We incorporated this change. We now specify that the measurements should be taken from a location downstream of the downstream block net. We also added to the water quality measurements for consistency throughout the document.

30. P33, L12 – Change settings to values.

    We changed settings to values.

31. P33, L34 – Change upstream to downstream.

    We made this change.

32. P35, L5 – Requiring netter to carry stopwatch could lead to decreased effectiveness of netter. Watching the clock, the water for fish, catching fish, staying clear of the backpack shocker, maintaining footing, carrying bucket for fish is a lot of activities. Again, consider alternative for watching the clock.

    The concern made by the reviewer is valid. However, the timing device is necessary. Variability in survey effort among passes will lead to heterogeneity in detection probability, and thus provide a negatively biased abundance estimate that does not reflect a species true abundance (Stewart et al. 2019). The number of "on-time" seconds is not easily seen given its location on the electroshocking unit. Often those conducting the survey are unaware of the number of "on-time" seconds during the survey. Therefore, and to limit the potential of the timing device to decrease effectiveness of the netter, we now specify that the timing device be placed on the inside wrist to ensure that the dial remains easily visible during each pass. Given that the electroshocking unit typically remains "on" throughout the duration of the pass, the number of seconds between the two devices should be approximate.

33. P35, L28 – Fish should be monitored immediately upon capture for signs of recovery. If they are not doing well in the bucket when released from the net then surveys should be halted and the shocking unit adjusted.

    We incorporated this change.

34. P35, L35 – Will subsequent passes be performed immediately or will there be a wait time to allow for sediment to clear for greater visibility?

    We incorporated this suggestion in SOP 1.

35. P36, L16 – Given that aerators will be used it is safe to hold fish in buckets until habitat is completed. I'd suggest holding them until then.

    We incorporated this suggestion. The fish will be held in the respected bucket until after habitat is measured.

36. P58, L3 – 15 is the maximum number of passes, data sheet should provide space to record that number of passes.

    The data sheet is correct. We plan to sample for no more than 10 passes. The maximum number of passes is changed from 15 to 10.

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